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**NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT**  
**ANNUAL REPORT OF INTRAMURAL RESEARCH**

October 1, 1986 through September 30, 1987

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT  
INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

1987

The Intramural Research Program is broadly concerned with the biological and neurobiological, medical, and behavioral aspects of normal and abnormal human development. In addition to four major clinical research and training programs in the areas of genetics and endocrinology, a diversity of developmental models are under study in twelve fundamental research Laboratories, drawing upon observations in bacteria, Drosophila, yeasts, viruses, molluscs, frogs, rodents, and subhuman primates. Disciplines employed in these studies include biochemistry, virology, molecular biology, immunology, pharmacology, genetics, cell and neuronal biology, biophysics, mathematical and theoretical biology, reproductive physiology, and comparative ethology. During the past year, we have emphasized a number of research programs that we believe have a particular promise and timeliness. In these areas, new resources have been added so that the investigators will be optimally poised to exploit their recent findings. These programs address problems in the cellular and molecular biology of development (growth and differentiation), wherein we anticipate significant results with broad implications for biomedical research. Indeed, it is our notion that the central, and arguably the most exciting, question in contemporary biology is: What are the mechanisms by which gene expression is developmentally regulated?

The Laboratory of Molecular Genetics (LMG), which employs a variety of prokaryotic and eukaryotic models to study gene transmission and expression, continues to expand its studies on the transgenic mouse, a powerful tool for illuminating the spatial and temporal regulation of genes. The 5' regulatory (promoter/enhancer) region of a crystallin gene has been used to direct expression of a marker transgene exclusively to the lens fiber cells, wherein crystallins are normally expressed. Moreover, this same crystallin regulatory segment has been employed to target the expression of an oncogene (SV40 T antigen) to the eye, resulting for the first time in "spontaneous" lens tumors. These results suggest the possibility of determining the molecular "switch" between differentiation and neoplasia, using this elegant and facile model. In another project, the 5' regulatory sequences from a murine milk protein gene were fused to a cDNA encoding human tissue plasminogen activator (TPA). Milk obtained from the resulting transgenic females was shown to contain biologically active TPA. Thus, in addition to its extraordinary power in elucidating basic mechanisms of gene regulation in vivo, the transgenic mouse may permit the production of medically useful foreign proteins on a large scale. Another transgenic mouse project involves certain aspects of the biology of HIV, the causative agent of AIDS. Mice carrying the regulatory (LTR) region of HIV linked to a marker gene, CAT, were found to express low levels of CAT, primarily in the thymus. This expression could be stimulated by mitogen treatment of the animal or coinfection with other viruses, suggesting that the transgenic mouse will prove to be an extremely useful model for the study of factors that affect the latency of HIV

and the onset of AIDS. Another group in the LMG has continued to explore gene expression in the developing frog embryo by the method of subtraction hybridization. These investigators have been able to identify genes which are among the earliest to be expressed by the embryo during its development. Interestingly, many of these genes, first expressed in the middle of blastula formation, encode keratins, fundamental structural elements of virtually all cells. In the past year, cloned keratin genes were injected into fertilized frog eggs, which should allow an analysis of the specific cellular proteins which bind to the keratin genes and regulate their expression in the earliest stages of frog development. This same group has also identified genes recently that appear to be specific for the earliest stages in development of the brain. The LMG has also progressed in its work on homeotic genes, which specify the spatial and temporal parameters of organ and limb morphology during development of the fruit fly, Drosophila melanogaster. It has become apparent that these very important genes, which play a critical role in normal embryogenesis, have been highly conserved throughout evolution, and homologous genes appear to be present in humans. LMG investigators have now cloned several homeotic genes and characterized their transcripts, which should permit a structural (and possibly a functional) prediction regarding the proteins they encode. Other LMG scientists are also concerned with the mechanisms that regulate gene batteries which demonstrate hierarchical and sequential interactions. One group focuses on the control of induction of amino acid biosynthetic enzymes in yeast by the availability of amino acids in the growth medium, and has now found that the proximal regulatory gene in the hierarchical cascade, an activator of many genes in multiple biosynthetic pathways, is regulated at the translational level--one of the first eukaryotic examples of this level of gene control. All of these studies are closely related to the central theme of the LMG, which is to illuminate the molecular mechanisms by which the expression of large batteries of genes is coordinately regulated such that global cell biologic events--growth, differentiation, and response to environmental stimuli--are carried out correctly, and at the right time in development.

The Cell Biology and Metabolism Branch (CBMB) is focusing on the development and regulation of various cellular organelles and receptors. Two groups are studying the expression of genes involved in the control of cellular iron metabolism; the regulated uptake and intracellular distribution of iron are critical to the metabolic health of all cells, and are intimately linked to cellular proliferation. These investigators have made great progress during the past year in studies on the transcriptional and translational regulation (by iron availability) of the genes which encode ferritin and the human transferrin receptor. These studies have led to the identification of a specific DNA sequence that encodes translational control in human cells. This translational control sequence has been fused to marker genes, enabling the construction of a new class of regulatable expression factors for eukaryotic cells. Much progress has also been made by another group on the T-cell antigen receptor (TCAR); this receptor is the most complex cell-surface receptor yet to be discovered in terms of subunit structure, and this lab is attempting to define TCAR structure through the isolation of genes encoding each of the subunits. They have also shown that antigenic stimulation of the TCAR leads to activation of two distinct phosphorylative signaling pathways which eventuate in T-lymphocyte proliferation and the immune response. Currently, T-cells from patients with AIDS are being studied with respect to these two signalling pathways. Preliminary evidence suggests a lesion in phosphorylation which might account for the lymphocyte nonresponsiveness in this disease. The CBMB's studies on the TCAR also present a unique opportunity to explore a complex organelle with respect to the stoichiometry of assembly of



the various components of the multi-component complex. Finally, in studies on interleukin 2 (IL-2) and the IL-2 receptor, the hormone-receptor system that drives the proliferation of activated human T-cells, another CBMB group has shown that the high affinity IL-2 receptor is composed of at least two subunits which may be differentially expressed in different types of lymphocytes. Further, this group has defined both the structure and regulation of the IL-2 receptor gene, demonstrating abnormal regulation in HTLV-I induced T-cell leukemias (which leads to the formulation of a specific molecular model for T-cell leukemogenesis).

The Human Genetics Branch (HGB) studies molecular aspects of human genetics and carries out clinical research on the natural history, diagnosis, and treatment of several heritable human disorders. During the past year, basic work by one group of investigators in this Branch has concentrated on the mechanism involved in the transport of RNA from eukaryotic nucleus to cytoplasm. At least in the case of tRNA, this transport appears to occur via a saturable, carrier-mediated mechanism involving a ribosome-like element at the nuclear envelope as the actual "motor." The nuclear envelope element appears to be rich in enzymes that process the RNA, with this processing closely linked to transport. This work on RNA transport utilizes frog eggs and led this year to the serendipitous discovery of a novel family of antimicrobial peptides isolated from the skin of the frog. This discovery has uncovered a previously unrecognized vertebrate host defense system, and the peptides involved may have therapeutic utility in human bacterial infections. Two major peptides, each 23 residues in length, have now been isolated and purified (the "Magainins"). The genes which encode the Magainins have also been identified and cloned, and the peptides themselves have been found to have a transmembrane, channel-like helical configuration, profoundly affecting membrane functions of susceptible organisms. Other work in the HGB is concerned with the structure and function of genes that may be defective in disorders of human bone formation, e.g., collagen genes in osteogenesis imperfecta. One lab is focusing on uteroglobin, a low molecular weight secretory protein that is steroid-inducible and has anti-inflammatory properties. Uteroglobin-secreting cell lines have been developed, as well as steroid-inducible vectors which express uteroglobin in *E. coli*. Other investigators in the HGB have utilized novel temperature-sensitive cell lines to study the molecular switches that control expression of marker genes during differentiation, e.g., the regulation of alpha-fetoprotein expression during liver maturation. Clinical research in the HGB focuses on disorders of bone formation and on human inborn errors of metabolism, especially disorders of lysosomal membrane transport. HGB investigators have defined the biochemical errors in both cytosinosis and Salla disease, the only human disorders known to be due to impaired transport of small molecules out of lysosomes. They have also defined a lysosomal transport system for tyrosine, and have shown that this carrier is responsive to thyroid-stimulating hormone. HGB investigators published an important report of a clinical trial this year demonstrating the efficacy of oral therapy with cysteamine in preventing fatal renal deterioration and improving growth in children with cytosinosis.

An area that has received much attention this year in the Laboratory of Developmental Neurobiology (LDN) relates to the cell biology of brain development, especially the hormonal and electrical events that endow the young mammalian brain with its rich but still not well appreciated structural and functional plasticity. Tools have become available to apply classical molecular biological techniques to the study of gene regulation in the developing nervous system, and this research is being pursued vigorously. The roles of specific



peptides in mediating activity-dependent neuronal survival during brain development have been described with considerable precision. LDN workers have now shown that communication between neurons and glia is mediated in part by vasoactive intestinal peptide (VIP), which after being released from neurons, stimulates receptors on glia, which then release other trophic factors for developing neurons. These results have permitted the construction of a neuropeptide model of neuron-glia-neuron interaction which seems to be of fundamental importance in our attempt to understand the molecular basis of neuronal survival during brain development. One group in the LDN recently demonstrated that the envelope protein (gp 120) of the AIDS virus (HIV) can produce neuronal death in culture, but this gp 120-induced death can be prevented by the addition of exogenous VIP; a remarkable sequence homology was found between VIP and gp 120. These investigators thus appear to have developed a valuable experimental model for studies on the mechanisms involved in the progressive dementia associated with AIDS. The LDN also continues to focus on membrane mechanisms related to synaptic transmission in cell culture systems which serve as models of the mammalian central nervous system. Much progress has been made on the characterization of postsynaptic receptors for excitatory amino acids. This work adds to the growing evidence that excitatory amino acids play a role in many diverse neuronal functions, including the programming of motor activity, the formation of memory, and cellular mechanisms that underlie neural pathological processes such as Alzheimer's disease. Much work has been carried out on the receptors for the acidic amino acids (L-glutamate and L-aspartate) that are thought to be the transmitters which signal excitatory messages across central synapses. Another group in the LDN is studying the mechanism by which neurotransmitters are secreted from nerve terminals. Modulation of the quantity of the transmitter released at the terminal may form the basis for all CNS functions, including the integration of information, as well as long-term information storage and retrieval. This group, which focuses on the neurohypophyseal, neuroendocrine cells as a model system, has demonstrated the importance of ionic channels on the initiation and modulation of secretion at the nerve terminal. In particular, a  $K^+$  channel seems to play a central role in secretory modulation caused by frequency information in the action potential train. These studies should permit the first direct biochemical identification of a  $K^+$  channel, as well as a molecular description of neuronal excitability. One group in the LDN uses the pineal gland as a model in studies on neural regulation of cell function and neural gene expression. This group is focused increasingly on the cell biology of the signal transduction mechanisms involved in catecholaminergic stimulation of the pinealocyte. During the past year, much progress has been made in elucidating the cell biologic mechanisms which couple neural activities to regulated gene expression, making the pineal an even more favorable model for gaining understanding of major neural processes.

The Laboratory of Neurochemistry and Neuroimmunology (LNN) is concerned with the development, functional organization, and interactions between the central nervous system and the endocrine system. The recent emphasis has been on the ACTH/endorphin/ $\alpha$ -MSH family of peptides, all of which are synthesized in the intermediate lobe of the pituitary from a common glycoprotein prohormone (POMC). This Laboratory has now isolated and purified several of the enzymes involved in the processing of this large prohormone, with particular interest in the prohormone converting enzyme (PCE). PCE is the first enzyme shown to process intact prohormones, and may be of considerable commercial value when used for the cleavage of precursors synthesized by bacteria which have been transfected with vectors that carry prohormone cDNA sequences. Cloning of the

PCE gene is underway. Other workers in the LNN are studying the regulation of POMC synthesis in the frog and mouse pituitary, with the finding that POMC synthesis can be independently regulated at the transcriptional and translational levels by stress, salt loading, etc. POMC-related peptides may also play a role in very early stages of neurogenesis; hence, the expression of POMC during CNS development in the mouse is being explored. Finally, LNN scientists are employing POMC as a model of regulated (versus constitutive) protein secretion, and have recently identified the sequences in the prohormone gene which are necessary and sufficient for entry of these peptides into the regulated pathway. Dr. Harold Gainer, Chief of the LNN, left the Institute in March 1987 and in the coming year, this Laboratory will be merged with the Laboratory of Developmental Neurobiology. Additionally, a new investigator, whose expertise is in neuronal molecular biology, will join the LDN from Washington University. It is planned to develop a research program in Drosophila neurogenetics as well as in the developmental regulation of myelin gene expression. With these moves, the Laboratory of Developmental Neurobiology, already a strong Laboratory for two decades, will undoubtedly continue to be one of the world's premiere laboratories for studies on the molecular and cell biologic aspects of the developing brain.

The Laboratory of Developmental and Molecular Immunity (LDMI) has put much effort this year into the development of a new generation of bacterial vaccines, the design of which is predicated upon an understanding of the development of the immune system in infants and children. In particular, the group responsible for these efforts has focused on new vaccines directed against pertussis, H. influenzae, Salmonella typhi, and pneumococcus--all agents with great consequences for the public health. During the past year, these investigators prepared a protein conjugate of the H. influenzae capsular polysaccharide and demonstrated in a trial in Swedish children that the conjugate is at least ten-fold more immunogenic than the polysaccharide vaccine alone. These results, now being expanded in Sweden as well as North Carolina, suggest that it should be possible to develop a conjugate vaccine for use in infants, who are the group at greatest risk for H. influenzae morbidity and mortality, but who are not protected by currently available vaccines. Another important achievement by LDMI investigators has been the development of a typhoid vaccine based upon the Vi capsular polysaccharide of S. typhi. A large trial in Nepal demonstrated that this new vaccine is at least as effective as all current typhoid vaccines, with many fewer side-effects and longer-lasting immunogenicity. A third vaccine now being brought to phase II trials by these investigators is a new pertussis vaccine composed of a single protein, pertussis toxin, inactivated to form a toxoid. This newly developed monovalent toxoid vaccine is the result of several years of painstaking and innovative work to improve the cultivation of the pertussis organism and the purification of its toxin. Phase I clinical trials have already demonstrated that the new vaccine is free of side-effects and is highly immunogenic. The new vaccine will be the subject of large clinical trials during the coming year in Sweden and in Massachusetts. At the more basic level, the LDMI is heavily involved in studies on the structure and regulation of genes which influence important immunological phenomena during development, such as the expression of class I major histocompatibility (MHC) antigens and the interferon system. During the past year, much progress has been made in studies on the regulation and structure of murine MHC antigens at the molecular level. Structure-function relationships of MHC proteins are being studied by site-directed mutagenesis. The domains of the MHC antigens responsible for interaction both with lymphoid cells and monoclonal antibodies have been identified, and much work has been accomplished on the three-dimensional



structure of MHC antigens. Because of the essential role of these antigens in immune responsiveness and resistance against infection and malignancy, these studies are critical for an understanding of immune function. LDMI workers have also defined two regulatory regions within class I MHC genes, a class I-specific regulatory element, and an interferon consensus sequence. DNA binding proteins which interact with these class I MHC regulatory sequences have also<sup>1</sup> been identified, and the mechanism by which they control class I MHC antigen expression is being vigorously pursued.

The Laboratory of Theoretical and Physical Biology (LTPB) has developed a number of new and novel applications of mathematical modelling, statistical analysis, mass spectrometry, and gel electrophoresis in the past year. LTPB investigators have placed particular emphasis on the characterization of receptors for vasopressin and oxytocin, opioid peptides, and the addictive drug, phencyclidine (PCP). The use of mathematical modelling has made it possible to resolve the subtypes of these receptors, and an improvement in the description of the receptor subtypes should assist in the development of new drugs (agonist and antagonist) with improved specificity for the desired receptor. For these studies, several practical computer programs were written to permit selection of the optimal concentrations of drug (ligand) with regard to the most precise estimates of binding capacity and affinity. Other workers in this Laboratory have made important advances in the application of mass spectrometry to the determination of compounds of biological and clinical interest. These workers have developed several important methods for analysis of carbohydrates, steroids, and their metabolites using thermospray liquid chromatography/mass spectrometry. These methods provide information on intact thermolabile molecules without resorting to chemical derivitization. Using this approach, this group has demonstrated that the normal cortisol production rate in man is only 50% of previously published estimates. This result is of immediate practical significance with respect, for example, to the therapy of Cushing's syndrome. The use of stable isotopic tracers has also permitted elegant studies on whole-body calcium metabolism, with the discovery of previously unrecognized slow turnover components of calcium flux. This finding promises to be of particular importance in the therapy of osteoporosis. Finally, the LTPB has continued its efforts in the development of algorithms and computer programs that guide the therapeutic management of chronic diseases such as diabetes mellitus. These programs have the potential to greatly refine treatment regimens, and they also encourage the patient with access to a personal computer to take a more active role in his own treatment. Moreover, the national data base being accrued through the use of these programs is clearly an invaluable clinical research tool.

Studies in the Endocrinology and Reproduction Research Branch (ERRB) are devoted to an elucidation of the cellular mechanisms involved in hormone secretion and action. This work includes studies on the characterization of peptide hormones and their cellular receptors; the structure-function relationships of peptide and glycoprotein hormones; the regulation of hormone synthesis and secretion; and the mechanisms of peptide hormone action within endocrine target cells. During the current year, much progress has been made in studies on the receptors and signal processing that are responsible for the control of steroid production in various endocrine target cells. Particular emphasis has also been given to the renin-angiotensin system and aldosterone secretion, and the role of protein phosphorylation in metabolic regulation. Major findings include the observation that GnRH stimulates the production of several higher inositol phosphates (IP<sub>3</sub>, IP<sub>4</sub>, IP<sub>5</sub>) in its pituitary target cells, further securing the details

of this signal transduction pathway. Complementary studies on angiotensin II (AII) receptors and their intracellular signalling pathways in the adrenal have also indicated the importance of the four-monophosphate pathway in inositol polyphosphate metabolism, and have revealed still other phosphorylation pathways and inositol metabolites with potentially important roles in intracellular signalling. The molecular basis of hormone action during cellular differentiation was explored using the ovarian granulosa cell as a model system, with emphasis on the functions of transforming growth factors as well as gonadotropins during granulosa cell development. Other investigators in the ERRB have purified the LH/hCG receptor to homogeneity, now confirmed by microsequencing. This group also found that receptor dimerization and probably further aggregation are necessary for signal transduction, and that receptor phosphorylation by one or more kinases may be involved in regulating gonadotropin action. These workers have also continued their studies on the measurement of plasma LH by biologic as well as immunologic assays, demonstrating that bioactive LH and immunoreactive LH may, in some clinically relevant settings, differ. Finally, one group of ERRB investigators studies the role of protein kinases and protein phosphorylation-dephosphorylation in the regulation of cellular functions. During the past year, these workers have focused on the signal transduction pathway associated with protein kinase C activation, a critical pathway which has been implicated in the regulation of cell growth, differentiation, gene expression, hormone release, neurotransmitter release, and possibly the induction of memory. This group has now been able to purify three types of protein kinase C isozymes to homogeneity, demonstrated the differential expression of these isozymes in various cell types (including various regions of the brain), and established that the protein kinase C isozymes are products of discrete genes. The presence of multiple kinase C isozymes may be essential to the broad variety of cellular functions in different tissues which are associated with this enzyme. The type I kinase C isozyme, which has been identified only in brain, was found to be selectively enriched in the temporal pole, lending further support to the notion that protein kinase C may be involved in higher mental functions such as learning and memory.

The Developmental Endocrinology Branch (DEB) undertakes clinical research involving human disorders of growth and development. Currently, DEB studies focus on problems in reproductive endocrinology, linear growth, and the role of the adrenal gland in development as well as in its link to homeostasis and the stress response. Significant results this year include findings on the human reproductive cycle which relate to ovulation and the mechanism by which a single egg is selected each month. Studies in which the potent progesterone antagonist, RU 486, was used as a probe, led to the conclusion that the signal responsible for ovulation probably is transmitted from the ovary to the pituitary. These results also suggest that some patients with "idiopathic" infertility may produce or process the progesterone signal abnormally. Other investigators in the DEB have been concerned with the structure and physiology of important glycoproteins such as hCG, and this year they have demonstrated that the beta core fragment is the unique metabolic product derived from a variety of hCG-related molecules of varying kinetic characteristics. Knowledge of how carbohydrate structure regulates hormone survival in the plasma and tissue distribution is clearly a key to the development of therapeutic analogs and to the diagnostic role of hormone measurement in pregnancy and in neoplastic disease. In studies on precocious puberty, DEB investigators have previously shown that a potent LHRH agonist can be used effectively in the treatment of gonadotropin-dependent central precocity. These studies are now being extended to the development of rational treatments for other variants of this disorder. Other workers in the DEB have been



concerned with linear growth and an attempt to define further the roles of growth hormone, growth hormone releasing hormone (GHRH), thyroxine, and somatomedin C (IGF-I). They have now shown that in many children with short stature, GHRH provides an effective alternative to growth hormone itself (which even in the genetically engineered form may be associated with antibody induction). Another group of DEB investigators has been concerned with the activation of the hypothalamic-pituitary-adrenal axis by stress and they have found that this stress-mediated activation is dependent on corticotropin-releasing factor (CRF). These researchers have recently found the same CRF dependency in alcoholism, depression, and in other psychiatric disorders associated with increased cortisol secretion, although Cushing's disease itself seems to be CRF-independent. These investigators have also demonstrated that CRF measurement is the test of choice for distinguishing between pseudo-Cushing's states and frank Cushing's disease. Using asymmetric CRF secretion to localize ACTH-secreting microadenomas, the DEB's cure rate for this disorder is now better than 95%, an extremely important clinical advance. RU 486, which is a potent glucocorticoid antagonist as well as a progesterone antagonist, is also being used in the therapy of Cushing's disease, and may be as effective as surgery in some patients. Finally, the DEB is devoting considerable effort to the development of a new therapy for pituitary tumors that depends upon boron-mediated neutron capture. Since pituitary tumors continue to display surface receptors for hypothalamic-releasing factors, the boron-linked factors can be delivered intravenously, and upon reaching the pituitary tumor cells, they will be internalized. Following external bombardment, the alpha-emitting boron should lyse these cells with an exquisite degree of specificity.

The Laboratory of Comparative Ethology (LCE), established four years ago, continues to undergo a major building program at the NIH's animal center in Poolesville, Maryland. An extensive outdoor facility for free-ranging primates has been established, allowing observational studies on the genetics of behavioral development. A three-floor indoor facility, including breeding quarters and a newborn nursery, was completed this year. The goal of this Laboratory is to integrate the results of behavioral research on humans as well as nonhuman primates, with this research directed toward a definition of the genetic components of stereotypical behaviors, and the influence of the environment on the genetic parameters of behavioral development. The comparative approach is utilized in order to define with precision the origin, ontogeny, and evolution of various behavioral phenotypes. These studies are consistently demonstrating clear differences among individual Rhesus monkeys with regard to their genetically determined response to minor environmental stress. These primates have the same behavioral phenotype in the absence of stress (e.g., brief separation from the mother), but in the face of challenge, two genotypes obtain: "uptight" and "laid-back." Moreover, these individual differences in response to challenge are clearly stable over long periods of major developmental change. Other work in the LCE is concerned with defining the heritability of characteristic patterns of social organization, and all of these studies on the genetic parameters of behavior not only demonstrate genotype-specific physiologic concomitants (e.g., plasma cortisol and CSF dopamine levels), but show defined genetic linkage patterns. Current studies in the LCE are directed toward examining how expression of the uptight and laid-back genotypes (under stress) can be modified as a function of the behavior of the particular mother rearing an infant with either of the behavioral genotypes; pharmacologic intervention; and the influence of peer-group rearing as opposed to mother-rearing. Other investigators in the LCE are concerned with the development of language and communication, with a particular focus on

species-specific vocalization as well as the heritability of specific vocalization patterns by individuals. The structure of monkey vocalization, emitted spontaneously in natural settings, is being modelled under laboratory conditions, and the nature of the neural chemical control of these vocal patterns is being characterized. Another LCE group is investigating the use of vocal signals by squirrel monkeys in complex social contexts, focusing on both the acoustical features of the vocalizations and their information content for different group members. These studies should permit the further development of an infrahuman primate model of "conversation." One major project in the LCE involves an analysis of the effects of primate social play, a behavior that is widespread amongst mammals and is thought to be important in human development, particularly with regard to later socialization. Two new senior investigators joined the LCE during the past year. Dr. Marc Bornstein, formerly Professor of Psychology at New York University, has become head of the LCE's Child and Family Research section, and will extend his previous research in the area of human infant perceptual and cognitive development. Bornstein's focus is on reliably identifying the genetic parameters of perception and cognition very early in infancy, and then defining the influence of various parental behaviors on the developmental outcome of cognitive and social competence. Dr. Michael Lamb, formerly Professor of Psychology at the University of Utah, will develop a new Section on Social and Emotional Development. Lamb's previous research has shown that the type of care offered to an infant has a much lesser impact on behavioral characteristics than the infant's intrinsic temperament. Much of this work has been accomplished in the context of cross-cultural comparison. Lamb also has a particular interest in the problems of adolescent parents, and has shown that both adolescent mothers and fathers have a history of diverse conduct disorders, such that pregnancy is merely one symptom of a complex behavior that defies simplistic attempts at intervention.

The Laboratory of Development Pharmacology (LDP) studies the molecular mechanisms of expression of genes which encode drug-metabolizing enzymes. This Laboratory has focused on the cytochrome P450-associated enzyme system, which is highly conserved from prokaryotes to man, and is fast approaching the development of molecular biology-based assays for determining the individual genetic polymorphisms which influence the function of this critical system. (The P450 enzyme system is responsible for the metabolism and detoxification of endogenous ligands such as bilirubin and steroids, as well as countless environmental chemicals which can cause birth defects, cancer, or idiosyncratic drug responses.) Intensive effort is being put into the cloning and sequencing of the many genes involved in the P450 system so as to develop useful probes for individual risk assessment, e.g., the risk of lung cancer faced by a given cigarette smoker. During the past year, LDP investigators have obtained further evidence in support of the proposition that the P450 gene superfamily is ancient, and has expanded via divergent evolution. In other work, these investigators have successfully transfected both yeast and mammalian cell cultures with P450 expression vectors, permitting studies on the regulation of expression of these important genes. The human P<sub>1</sub>450 and P<sub>3</sub>450 genes and their flanking regions have now been cloned, sequenced and localized to chromosome 15. Restriction fragment length polymorphisms (RFLP) have been found in relation to these genes, and families with high and low cancer incidence are now being studied. In addition to the first phase of P450-mediated drug metabolism, which can eventuate in the production of carcinogens and mutagens, a second phase of drug metabolism involves conjugation, such as in the case of the UDP-glucuronosyltransferase system. A number of cDNA clones which encode different forms of this



transferase have now been isolated and sequenced. The expression of transferase mRNA has been examined with regard to tissue and developmental regulation; genomic and cDNA clones are being utilized in a study of the regulation of each transferase as a function of age, tissue distribution, and treatment with prototypic inducers.

Finally, in the laboratories of the Scientific Director and Deputy Scientific Director, one project involves nerve growth factor (NGF), a polypeptide required for the survival and development of many types of neurons. NGF binds to specific cell-surface receptors and initiates a chain of intracellular events which leads to the expression of specific genes. Recent work has demonstrated that the binding of NGF to its receptor is followed rapidly by an activation of phosphoinositide metabolism as well as protein kinase C. This activation in turn leads to changes in the phosphorylation state of a number of key proteins within the cell, and several of these substrates have now been identified. An inhibitor has been found that is specific for the actions of NGF and interestingly, this inhibitor blocks the induction of the oncogene, c-fos, by NGF. Current work is directed toward determining which other genes are controlled by NGF, and how they may be integrated in the overall control of neuronal differentiation and cell division. Another project relates to antimitogenic growth factors, the products of putative "anti-oncogenes." This year, it was found that SV40-transformed rodent cells (which are highly oncogenic when inoculated into susceptible rodent hosts), but not adenovirus 2-transformed cells (which are nononcogenic), secrete a mitogenic inhibitor (MI) that strongly inhibits the proliferation of normal rodent cells but not transformed cells. Moreover, this MI is a powerful inhibitor of T and B lymphocyte proliferation, suggesting that the MI might contribute to the high oncogenicity of SV40 transformed cells by interfering with mobilization of immunoeffector cells at the site of tumor growth. In other studies in these laboratories, the processes of mammalian mutagenesis are being characterized, using an SV40-based shuttle vector as a probe to investigate the molecular mechanisms by which agents that damage DNA induce mutations in mammalian cells. Through use of the shuttle vector, the types of mutations that occur in mammalian cells, either spontaneously or in response to DNA damage, have been characterized extensively. An analysis of the sequence specificity of these mutations has led to a model which explains how the mammalian DNA polymerase introduces errors during DNA synthesis, causing mutations. Current studies with the shuttle vector utilize an in vitro DNA replication system which should permit a characterization of the cellular factors, in addition to DNA polymerase, that influence replication fidelity.

The Institute continues to acquire additional laboratory space. Construction of a three-floor addition to Building 6 was completed this year, and has provided 25 new laboratory modules as well as an extensive animal facility. This new space accommodates the Laboratory of Developmental Pharmacology, one section of the Laboratory of Developmental and Molecular Immunity, and the DNA/protein Sequencing and Synthesis Unit of the Endocrinology and Reproduction Research Branch. Currently, new space is being renovated in Building 10 for the Laboratory of Theoretical and Physical Biology which will allow all of the LTPB's investigators to be housed in one location. Within the neuroscience complex (Buildings 36 and 37), we have been able to add 25 additional modules for neurobiology research. This space is now being renovated and should be ready for occupancy in 1988. The Congress has appropriated 1988 funds that will permit still another addition to Building 6 which would house a large transgenic mouse facility as well as 8000 sq. ft. of additional laboratory space. The Congress also



appropriated \$10,000,000 for further work on a new building ("Building 49") on the NIH campus that would allow continuing expansion of the NICHD neuroscience and neuroendocrinology laboratories. This building will also house the neuroscience laboratories of two other Institutes as well as a state-of-the-art primate facility. Finally, NIMH has transferred a small building to us (Building 32), adjoining the CBMB, which will allow further expansion of NICHD's labs in that area of the campus. We anticipate that the NICHD intramural program will have grown from 41,000 sq. ft. of laboratory space (in 1983) to 100,000 sq. ft. by 1990.

In major personnel changes during the past year, Dr. Warren Leonard was granted tenure in the Cell Biology and Metabolism Branch, Dr. Alan Hinnebusch in the Laboratory of Molecular Genetics, and Dr. Douglas Brenneman in the Laboratory of Developmental Neurobiology. As noted previously, Drs. Mark Bornstein and Michael Lamb were recruited from New York University and the University of Utah, respectively, as new Section Heads in the Laboratory of Comparative Ethology. Dr. James Sidbury, a former Scientific Director of the Institute and more recently a section head in the Human Genetics Branch, retired in 1987, but will continue his research association with the Human Genetics Branch on a part-time basis.

Our clinical and laboratory research fellowships for physicians in adult, pediatric, and reproductive endocrinology, as well as the fellowship in medical genetics, continue to thrive, and in the past year, we developed new sources of support for postdoctoral (Ph.D. and M.D.) fellows. These mechanisms include postdoctoral stipends paid by the Institute but which do not encumber regular government positions. These new fellowships are greatly enhancing our ability to offer postdoctoral training, especially for American graduates, and include an Intramural National Research Scholarship Award (NRSA) for M.D.'s, a contractual program for the support of intramural training in biotechnology administered by the National Research Council of the National Science Foundation, and a new program that is intended for American postdoctoral candidates in any of the biological sciences (Ph.D.'s or M.D.'s). The latter mechanism (Intramural Research Training Award, IRTA) is the domestic equivalent of our long-standing Visiting Fellow program for postdoctoral candidates from abroad. We have also been successful in identifying new donors of stipends, including endowments by private industry and foundations. NICHD funds administered by the NIH's Fogarty International Center have been employed for the support of sabbatical visits by senior scientists from abroad. Moreover, a number of foreign postdoctoral fellows have been awarded stipends for training in our laboratories under the terms of formal bilateral agreements between the NIH and countries in Europe, Asia, and the Middle East. Finally, a number of medical students supported by the new Howard Hughes Foundation program at the NIH are working in our laboratories during an elective year. One such student, Katrin Andreasson, who spent a year in the Laboratory of Neurochemistry and Neuroimmunology, received the Alfred Steiner Award (on the basis of this work) for "the most outstanding research project undertaken by a student" at Columbia University's College of Physicians and Surgeons. Our summer student program during 1987 was extremely successful, with more than 60 undergraduate, graduate, and medical students working in our laboratories.

Peer review of intramural research, conducted by the Institute's Board of Scientific Counselors and ad hoc experts, continues to receive great emphasis, with rigorous site visits to each Lab at four-year intervals. During 1987, visits were made to the Laboratory of Molecular Genetics, the Developmental Endocrinology Branch, and the Endocrinology and Reproduction Research Branch,

with detailed critiques prepared as a consequence of these visits. The membership of the Board of Scientific Counselors reflects the increasing diversity of research interests within this intramural program, and currently includes Joseph G. Gall, Ph.D. (Chairman), Senior Member, Carnegie Institution (Baltimore); Stanley Cohen, Ph.D., Professor of Biochemistry, Vanderbilt University; Lewis P. Lipsitt, Ph.D., Professor of Psychology, Brown University; Allen H. Neims, M.D., Ph.D., Professor and Chairman, Department of Pharmacology and Therapeutics, University of Florida; Story C. Landis, Ph.D., Professor of Pharmacology, Case Western Reserve University; Merry R. Sherman, Ph.D., Professor of Biochemistry, Rutgers University; John A. Phillips, III, M.D., Professor of Pediatrics and Human Genetics, Vanderbilt University; Stanley G. Nathenson, M.D., Professor and Chairman, Department of Microbiology and Immunology, Albert Einstein School of Medicine; and Peter O. Kohler, M.D., Dean of the Medical School, University of Texas Health Science Center.

Other developments in the past year include the continued strengthening of all aspects of the care and use of animals employed in our research. Dr. William Stokes was recruited from the U.S. Army Medical Research Command to become the Institute's Chief Veterinarian, and Dr. Nelson Garnett became the veterinarian at our Poolesville facility. We have continued to develop new computer-based administrative procedures in the Office of the Scientific Director so as to maximum the efficiency with which our resources are deployed. These new administrative approaches are ensuring the maximum yield with respect to scientific productivity while the current climate of constrained resources persists. A number of laboratories within the program are involved in various aspects of research on AIDS; they competed successfully for 1987 special set-aside funds for AIDS research above the Institute's normal budget ceiling, and it is likely that this budget increment will be continued for the foreseeable future.

Seminars by the 12 Laboratories and Branches in this program were numerous and well attended throughout the year, such that this Institute organized a relatively large fraction of the NIH's overall offering of intramural seminars and workshops. During the past year also, three major international conferences were organized by Laboratories of the intramural research program on the Bethesda campus including: "The Second International Workshop on P450 Gene Regulation," hosted by the Laboratory of Developmental Pharmacology; "Gene Expression and Early Nervous System Development" (Laboratory of Developmental Neurobiology); and "Gene Regulation in the Developing Immune System" (Laboratory of Developmental and Molecular Immunity and the Office of the Scientific Director).

During the year, we were especially honored by the choice of Dr. Igor Dawid (Chief, Laboratory of Molecular Genetics) to present the NIH's Annual Mider Lecture, one of only two named lectureships at this Institution. Dr. Maria Dufau received the NIH Director's Award for the body of her work in cellular and molecular endocrinology, and Dr. Arthur Levine received the Public Health Service Meritorious Service Medal for his scientific leadership. Dr. Michael Zasloff (Chief, Human Genetics Branch) received the Public Health Service Commendation Medal for his pioneering work on RNA transport. Dr. George Chrousos received the Richard E. Weitzman Memorial Award, presented by the American Endocrine Society for outstanding research achievements by a young investigator, and Dr. Kevin Catt was honored by his appointment as a Visiting Professor of the Royal Society of Medicine (London) and keynote lecturer of the British Endocrine Society's Annual Meeting. Dr. Stephen Suomi was elected a Fellow of the American Association for the Advancement of Science for his major contributions

to an understanding of the factors that influence the psychological development of nonhuman primates. Additionally, many of the Institute's senior investigators held honorary lectureships and visiting professorships during the year; other awards for research accomplishments were given to a number of our scientists by various universities and societies. Finally, during 1987 we sponsored two distinguished senior scientists in the Fogarty Scholars-in-Residence Program, Professor Donald Brown of the Carnegie Institution (Baltimore), and Professor Itzhak Parnas of the Hebrew University (Jerusalem) who worked in our laboratories during their sabbaticals.

In 1982 this Institute embarked upon an ambitious program designed to improve the quality and quantity of its intramural scientific productivity, even as the rate of growth in research support declined. As one reflection of our success in this regard during the past five years, NICHD intramural scientists this year published more than twice the number of peer reviewed original scientific reports, appearing in journals of stature, than had been the case prior to the beginning of this new direction. A rank order survey of the various NIH Institutes with respect to their yearly number of publications in nine of science's most prestigious journals included NICHD among the top three. Moreover, more than half of our postdoctoral scientists are now fully supported by a stipend awarded from a non-NIH source--reflecting, we believe, the current quality and productivity of this intramural program.

Arthur S. Levine, M.D.  
Scientific Director  
National Institute of Child Health  
and Human Development



## CELL BIOLOGY AND METABOLISM BRANCH

- Z01 HD 01600-03    Biochemical Basis of T Cell Activation  
                         Larry E. Samelson, M.D.
- Z01 HD 01601-03    Molecular Aspects of the Regulation of the Human  
                         Transferrin Receptor  
                         Joe B. Harford, Ph.D.
- Z01 HD 01602-03    Regulation of Intracellular Iron Metabolism  
                         Richard D. Klausner, M.D.
- Z01 HD 01604-02    Interleukin-2 Receptor - Structure, Function,  
                         and Regulation  
                         Warren J. Leonard, M.D.
- Z01 HD 01605-01    T-Cell Antigen Receptor - Structure, Biosynthesis  
                         and Cell Biology  
                         Richard D. Klausner, M.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Cell Biology and Metabolism Branch

The past year has witnessed significant progress in all of the areas of research in the Cell Biology and Metabolism Branch. The organization of the laboratory consists of four independent groups. Two groups study mechanisms of post-transcriptional regulation of gene expression in human cells. Both of these groups are utilizing specific genes involved in the regulation of cellular iron metabolism. The regulated uptake and intracellular distribution of iron is absolutely critical to the metabolic health of all cells and is intimately tied to cellular proliferation. One group, headed by Richard Klausner, studies translational control and the function and expression of the gene for ferritin. The second group, headed by Joe Harford, examines post-transcriptional and transcriptional regulation of the human transferrin receptor gene. Both of these regulatory processes determine the level of mature mRNA encoding this receptor. Two other groups in the branch are studying the molecular, biochemical and cellular basis for the activation and proliferation of cells of the immune system. One of these groups headed by Richard Klausner and Lawrence Samelson studies the role of the T cell antigen receptor in the development, activation and regulation of T cells. The other group, headed by Warren Leonard, has studied the structure and regulation of the interleukin-2 (IL-2) receptor in human T cells. This receptor is responsible for the proliferation of T cells during the immune response.

Translational Control and the Human Ferritin Gene -

One year ago, after the successful molecular cloning of the complete gene for human ferritin H chain, this group was poised to test the decade old hypothesis that the dramatic regulation of ferritin by iron occurred via translational control. Although strong evidence pointed to such a mechanism, this evidence was indirect. Using recombinant DNA techniques, we demonstrated that normal full length human ferritin protein could be produced in murine cells and be fully regulated by iron but only if the 5' untranslated sequences were present in the mRNA. Removal of these sequences resulted in the production of ferritin that was no longer biosynthetically responsive to iron. To prove that the removed sequence contained sufficient information to encode translational regulation, hybrid genes were constructed that would encode mRNA molecules containing the 5' untranslated region of ferritin but the protein coding region of unrelated genes. Expression of these hybrid mRNA molecules resulted in the



transfer of iron-sensitive translational control to other structural genes. The sequences responsible for this regulation were identified by deletion analysis of the 5' ferritin leader sequence. This predicted that a relatively short sequence, highly conserved in the evolution of ferritin genes, contained the genetic information for this control. To prove this we synthesized an oligonucleotide encoding the implicated region and with this were able to reconstruct translational control from these synthetic molecules. This work takes us a long way towards understanding how this key gene in iron metabolism is homeostatically controlled by the cell. But in addition to its implications for cellular iron metabolism, this work sheds new insights and provides new tools for the study of the broader problem of the control of mRNA translation in human cells. First, this represents the first time that a specific sequence has been defined that encodes translational control in human cells. Translational control is widely used by eukaryotic cells but the mechanisms underlying this important form of gene regulation remain entirely unknown. This discovery gives us the first highly specific route to begin to elucidate this problem. Second, the ability to transfer translational control to other genes enables us to construct a new class of regulatable expression vectors for eukaryotic cells. The lack of reliable regulatable expression systems is a major drawback in recombinant DNA techniques. The ferritin sequence should be unaffected by the stable incorporation of the gene into the chromosome and can be regulated by manipulations of iron that are effective while being non-toxic.

#### Regulation of the Human Transferrin Receptor Gene -

The rapid and wide range regulation of expression of this cell surface receptor is the means by which eukaryotic cells attempt to regulate their uptake of iron. Because of the essential role of iron for DNA synthesis, proliferating cells and cells stimulated to proliferate must make large numbers of these receptors. As with the ferritin gene, recombinant DNA techniques have been used to identify the transcriptional elements involved in the expression of this gene and have been used to uncover a region related to iron regulation contained within the portion of the gene that encodes the 3' untranslated region of the mRNA. This region, which shares homology with the translational control sequences in the ferritin mRNA, likely allows iron to determine the processing and/or survival of receptor mRNA. This discovery provides a superb model system with which to study the post-transcriptional regulation of mRNA levels.

#### Receptors and the Immune System

The group studying the T cell antigen receptor (TCR) has made great progress over the past year in three areas: 1) receptor

structure; 2) signal transduction; and 3) cell and organelle biology. This receptor, in part via the discoveries made in this laboratory, is the most complex known cell surface receptor in terms of subunit structure. All T cells that we have examined contain two classes of receptor. The majority consists of seven subunits including the two-component recognition subunit whose sequence varies for each T cell clone and five chains that are constant in all T cells. These include two glycoproteins ( $\delta$  and  $\gamma$ ), one monomeric protein ( $\epsilon$ ) and one dimeric protein ( $\zeta$ ). In addition, about ten to twenty percent of receptors contain an additional protein ( $\eta$ ) which has been biochemically characterized. It is a 21 KD non-glycosylated protein that is disulfide linked to  $\zeta$ . Thus  $\zeta$  can exist either as a homodimer or as part of a heterodimer. In order to understand the structure of the receptor, it is necessary to isolate the genes encoding each of the subunits. Recent sequence information on the  $\zeta$  protein has allowed us to identify cDNA clones encoding this subunit.

Signal transduction via cell surface receptors is one of the most fundamental and important of cellular processes. Through this, the regulation of cellular function via external signals take place. There has been a recent evolution in our thinking about receptor-generated signals exemplified by the work from this laboratory on the TCR. This evolution accompanies the demonstration that receptors are linked not to one but often to several biochemical signal generating systems. Thus we demonstrated that stimulation of this receptor leads to the activation of phosphatidyl inositol metabolism and to the activation of a tyrosine kinase. There are a variety of stimuli that result in the activation of these two pathways including specific antigen, anti-receptor antibodies and antibodies directed against certain other surface molecules on T cells. A surprising result emerged from studies in which the ability of cAMP to uncouple the TCR from these activation pathways was examined. This treatment differentiated between the different stimuli in terms of their ability to activate the tyrosine kinase pathway in the presence of cAMP. That different stimuli can be so distinguished tells us that not all stimuli trigger the identical biophysical events in the receptor and open up the possibility of partial or differential agonists for this receptor system. There are two classes of tyrosine kinases in cells. In one, exemplified by growth factor receptors, the kinase is contained within the primary sequence of the receptor chain. In the other, exemplified by the src-like kinases, the kinase, while often membrane associated, is not covalently linked to a receptor. The kinases of the first class is activated by receptor occupancy. How the second class of kinases is activated physiologically remains a complete mystery. The TCR possesses no detectable intrinsic kinase activity. Thus this receptor

represents the first example of the activation of a non-receptor tyrosine kinase being activated by a receptor. A new signal transduction pathway will likely emerge from this system and one that will give some insight into the regulation of tyrosine kinases. It is becoming clear that the T cell surface is populated by a large number of molecules that either can interact with the TCR or can influence the ability of the TCR to transduce signals. How these different molecules modulate and regulate the immune response is being studied at the biochemical level. The definition of the biochemical pathways of normal signal transduction involved in the activation of T cells opens the possibility of defining defects in these pathways. In order to apply this to human disease states, we had to demonstrate these pathways in lymphocytes taken directly from human blood. To this end the dual TCR activation pathways were recently demonstrated in these cells. Currently, T cells from patients infected with the human immunodeficiency virus are being studied with respect to TCR signalling pathways.

The complexity of the TCR has given us the opportunity of studying the biosynthesis, assembly, processing, intracellular transport and degradation of this complex in an attempt to explain how only the complete complex is expressed on the cell surface. The different subunits are not made in equal amounts. Early after synthesis in the RER, subunits begin assembling, a process that takes up to thirty minutes to complete. The subunits synthesized in the largest amounts are present in excess. The unassembled or incompletely assembled chains that result from this excess are efficiently destroyed by being sorted from the Golgi to lysosomes. These data provide a generalizable model for obtaining correct stoichiometry for multi-component membrane complexes. According to this model, individual chains contain signals that sort them to lysosomes. The process of assembly interferes with this so that completely assembled complexes are transported to the plasma membrane while any excess chains are destroyed. This obviates the need to have both coordinate synthesis of all chains and complete assembly of those synthesized chains.

Two observations about the fate of newly synthesized receptor subunits have opened additional avenues of investigation. First when certain subunits are expressed in murine fibroblasts they are rapidly degraded. This degradation is quite different than the lysosomal pathway taken by excess chains in the T cell. It appears to be either occurring in the ER or in a previously unrecognized organelle which defines a degradative compartment into which newly synthesized proteins may be sorted. The identification of this novel degradative pathway is ongoing. Careful examination of the immediate events after the biosynthesis of new receptor subunits in the ER in T cells has



demonstrated the presence of a protein that transiently associates with several of the chains. This protein which we have called TRAP (T cell Receptor Associated Protein) remains associated with the newly synthesized chains for about twenty minutes accompanying the chain to the Golgi where it dissociates, a process requiring energy and probably acidic pH. This protein may represent the first example of a new class of intracellular proteins involved in the correct intracellular transport of membrane components.

The group headed by Warren Leonard focuses on the hormone-receptor system that drives the proliferation of human T cells. In particular they are studying the biology of the interleukin-2 (IL-2) receptor. Progress over the past year has been outstanding in this new lab in two areas. With the first published report on a second subunit ( $\beta$ ) of this receptor, the biology of the IL-2 receptor has come into an entirely new light. That the high affinity receptor is composed of at least two subunits allows an entirely new set of questions to be asked about the regulation and function of this growth factor receptor. Unexpectedly in resting T cells and in large granular lymphocytes which can respond to high doses of IL-2 but lack the classical IL-2 receptor, the  $\beta$  subunit is present and is capable of binding IL-2. Thus the discovery of this protein represents not only a second subunit of a recognized receptor but a distinct receptor in its own right. This is the first example of such a phenomenon and has major implications for the activation of lymphocytes. Recent studies in collaboration with Jay Siegel (FDA) suggest that this new IL-2 receptor is responsible for the induction of natural killer cells and lymphokine activated killer (LAK) cells. Both of these cells may possess important clinical potential in the treatment of malignancies. It has long been known that certain cells of the immune system respond to IL-2 in the apparent absence of the IL-2 receptor. The finding of this second IL-2 receptor on these cells resolves this long-standing puzzle.

The IL-2 receptor gene is tightly and dramatically regulated during the immune response. This occurs largely at the transcriptional level. Importantly, in a variety of T cell leukemias caused by infection with the HTLV-I retrovirus, the IL-2 receptor expression is high and unregulated. The basis for this phenomenon which may be critically involved in abnormal T cell proliferation is unknown. Recently Dr. Leonard and his colleagues have characterized in detail the transcription control elements involved in the expression of the IL-2 receptor gene. They have defined a region of the gene that is sensitive to a factor present in HTLV-I infected cells. Furthermore they have directly demonstrated the ability of a retroviral gene product to induce the expression of the receptor gene. This has allowed the

formulation of a specific molecular model for T cell  
leukemogenesis.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01600-03 CBMB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Basis of T Cell Activation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	L. E. Samelson	Senior Staff Fellow	CBMB, NICHD
Others:	R. D. Klausner	Head	CBMB, NICHD
	A. M. Weissman	Medical Staff Fellow	CBMB, NICHD
	J. J. O'Shea	Senior Staff Fellow	CBMB, NICHD
	Y. Minami	Visiting Fellow	CBMB, NICHD
	M. Baniyash	Visiting Fellow	CBMB, NICHD
	H.T. Luong	Chemist	CBMB, NICHD
	P. Ross	Adjunct Scientist	CBMB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.9

## PROFESSIONAL:

2.9

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

T lymphocytes initiate and regulate the complex immune reaction of the organism to foreign antigens. The T cell response begins when antigen receptors on the surface of T cells engage antigen in the context of surface molecules encoded by the major histocompatibility complex. This antigen receptor is a multicomponent cell surface complex consisting of as many as nine chains. Receptor occupancy leads to rapid activation of two protein kinase pathways and subsequent phosphorylation of the receptor and other cellular substrates. The approach of the laboratory has been to completely characterize the receptor on a murine T cell hybridoma with antibodies binding the component chains. Work has been extended to biochemical analysis of receptors on normal human and murine T cells.

Characterization of protein kinase activation and regulation, especially activation of a protein tyrosine kinase has been extensively studied in order to understand signal transduction in normal T cells and those isolated from murine and human disease states.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01601-03 CBMB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Aspects of the Regulation of the Human Transferrin Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. B. Harford Senior Investigator CBMB, NICHD

Others: R. D. Klausner	Head	CBMB, NICHD
J. Casey	IRTA Fellow	CBMB, NICHD
B. Di Jeso	Visiting Fellow	CBMB, NICHD
D. M. Koeller	Medical Staff Fellow	CBMB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL:

2.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Iron is an essential element in cellular metabolism. Eukaryotic cells acquire iron through the endocytosis of diferric transferrin mediated by high-affinity transferrin receptors located in their cell membrane. The synthesis, degradation, and cellular dynamics of the transferrin receptor are highly regulated phenomena. In particular, the rate of receptor biosynthesis is altered depending on the state of differentiation and growth of cells. In a proliferating population of cells, the biosynthesis of the transferrin receptor is highly regulated by iron availability. The provision of iron to cells via hemin, inorganic iron salts, or diferric transferrin leads to decreases in the biosynthesis of the receptor. In contrast, intracellular iron chelation results in marked increases in receptor biosynthesis. Modulation of biosynthesis in both directions is a manifestation of corresponding changes in the level of mRNA encoding the receptor. Using the techniques of molecular biology and gene transfer, we are seeking to understand expression and regulation of the transferrin receptor. The promoter region of the receptor has been molecularly cloned and characterized. An enhancer-like element was identified approximately 75 bp upstream of the mRNA start site. A relatively low degree of regulation is conferred by the sequences upstream of the structural gene for the receptor. Rather, the sequences corresponding to the 3' untranslated portion of the mRNA have been implicated as the major locus of iron regulation.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01602-03 CBMB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Intracellular Iron Metabolism

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. D. Klausner Head CBMB, NICHD

Others:	M. Hentze	Adjunct Scientist	CBMB, NICHD
	T. A. Rouault	Medical Staff Fellow	CBMB, NICHD
	S. Wright Caughman	Adjunct Scientist	CBMB, NICHD
	Andrew Dancis	Medical Staff Fellow	CBMB, NICHD
	Javier Barriocanal	Visiting Fellow	CBMB, NICHD
	J. B. Harford	Senior Investigator	CBMB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.1

## PROFESSIONAL:

3.1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

The molecular biology of intracellular iron metabolism has been studied by examining the regulation and expression of the gene for human ferritin. Ferritin functions to store, detoxify and regulate intracellular iron. It performs all of these functions via its ability to accumulate large amounts of iron within a 24 subunit shell. The critical determinant of the effect of ferritin upon the cell is its concentration. This is determined by both the level of expression of the genes encoding ferritin and, by iron, through the level of biosynthesis of the protein. We have isolated the gene for human ferritin H chain and have analyzed the molecular basis for the regulation of ferritin biosynthesis by iron. We have shown that it is the 5' untranslated region of the ferritin mRNA that contains the information for translational control by iron. This control can be transferred to other structural genes by creating hybrid mRNA molecules with the ferritin leader sequence 5' to the coding sequences. This region has been mapped by deletion analysis to a short region that is highly conserved among all known ferritin leader sequences. A 26 nucleotide molecule was synthesized which, when linked 5' to the translation initiation codon, confers iron dependent translational control. The molecular mechanism whereby these 5' mRNA sequences control translation are being examined in order to understand the regulation of gene expression in human cells.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 HD 01604-02 CBMB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Interleukin-2 Receptor - Structure, Function, and Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Warren J. Leonard	Senior Staff Fellow	CBMB, NICHD
Others:	Michael Sharon	Medical Staff Fellow	CBMB, NICHD
	James Gnarr	IRTA Fellow	CBMB, NICHD
	Sharon L. Cross	Adjunct Scientist	CBMB, NICHD
	Julie B. Wolf	Guest Technician	CBMB, NICHD
	Nancy Halden	Guest Technician	CBMB, NICHD
	Myra Lipis	Medical Staff Fellow	CBMB, NICHD
	Monica Napolitano	Visiting Fellow	CBMB, NICHD

## COOPERATING UNITS (If any)

Nancy Chang, Baylor College of Medicine, Houston, TX; Richard Chizzonite, Hoffmann La Roche, Inc., Nutley, NJ; Flossie Wong Staal, Lab of Tumor Cell Biology, NCI; Jay P. Siegel, Division of Virology, FDA

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS.

3.3

## PROFESSIONAL:

3.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human interleukin-2 (IL2R) is being studied in order to understand specific critical components of the T cell immune response in normal and neoplastic cells. The approaches used are based on (1) biochemical analysis of high and low affinity IL2Rs; (2) identification of key transcriptional regulatory sequences in the IL2R gene; (3) identification of DNA binding proteins for regulatory regions; (4) analysis of the importance of post-transcriptional regulation for IL2R gene expression. We have identified a 65 to 77 kD glycoprotein component of the high affinity human IL2R, distinct from Tac antigen. This protein, denoted p70 or the IL2R beta chain, is itself an IL2 receptor, independent of its association with Tac antigen. We have demonstrated that p70 appears to mediate both the generation of lymphokine activated killer cells and IL2R-induced augmentation of natural killer cell activity. It is present on resting CD4 and CD8 positive T cells, and can be induced on B cells and monocytes. Using IL2R cDNA and genomic constructs, we have partially mapped the region of the IL2R gene necessary for transcriptional activity. We have identified: (1) a region functioning as an IL2R negative regulatory region in HTLV-I transformed T cell lines; (2) a requirement for a larger promoter region in Jurkat cells than in HTLV-I transformed T cells; (3) the ability to utilize a smaller promoter in Jurkat cells, analogous to HTLV-I transformed T cells, by cotransfection with tat-I; (4) several regions of DNA that putatively bind proteins based on exonuclease assays and band shift experiments. We have also identified a new gene, Act2, absent in resting T cells but induced with 30 minutes of exposure to PHA, reaching maximal levels by 4 hours and then significantly falling by 16 hours.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01605-01 CBMB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. D. Klausner	Head	CBMB, NICHD
Others:	Lawrence E. Samelson	Senior Staff Fellow	CBMB, NICHD
	Yasuhiro Minami	Visiting Fellow	CBMB, NICHD
	Allan Weissman	Medical Staff Fellow	CBMB, NICHD
	Michal Baniyash	Visiting Fellow	CBMB, NICHD
	Juan Bonifacino	Visiting Associate	CBMB, NICHD
	Jennifer Lippincott-Schwartz	PRAT Fellow	CBMB, NICHD
	Pilar Garcia Morales	Visiting Fellow	CBMB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

5.2

## PROFESSIONAL:

5.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The specific activation of T lymphocytes which govern the immune response occurs via stimulation of the T Cell Antigen Receptor. This is a multichain and multimorphic receptor existing as either a seven or nine chain transmembrane complex. In most mature T cells, the ligand specificity is imparted by two chains,  $\alpha$  and  $\beta$ , which exist as a heterodimer. In all T cells these chains are non-covalently linked to four other membrane proteins,  $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$ - $\zeta$  homodimer, as a seven-chain complex. In all T cells studied, we have found that 10% of the cell receptors possess a  $\zeta$ -p21 heterodimer in addition to or instead of the  $\zeta$ - $\zeta$  homodimer. The structure, initially described in murine T cells, is identical in human T cells. Mutant or variant T cells with absent and/or abnormal subunits are studied to illuminate the structural and functional roles of individual receptor components. The cycling dynamics of the cell surface T cell receptor have been studied and kinase-mediated perturbations examined. The process of biosynthesis, maturation, assembly, degradation and intracellular routing of receptor components are studied both in T cells and in fibroblasts expressing individual genes or sets of genes encoding these subunits. Models for the relationship between assembly and intracellular sorting have been developed. Previously unanticipated routes of intracellular degradation (and sorting) have been suggested by these studies. A new protein has been identified which may play a specific role in the targetting and/or assembly of newly synthesized receptor chains.





## DEVELOPMENTAL ENDOCRINOLOGY BRANCH

- Z01 HD 00610-07 Puberty and its Disorders: Physiology, Pathophysiology  
and Therapy  
Gordon B. Cutler, Jr., M.D.
- Z01 HD 00613-07 Clinical and Basic Studies of Male Reproduction  
Richard J. Sherins, M.D.
- Z01 HD 00614-07 Biology of Hormone Binding Proteins  
Bruce C. Nisula, M.D.
- Z01 HD 00615-07 Steroid Antagonists  
George P. Chrousos, M.D.
- Z01 HD 00616-07 Structure, Function, and Physiology of Glycoprotein  
Hormones  
Bruce C. Nisula, M.D.
- Z01 HD 00618-06 Physiology and Pathophysiology of the Hypothalamic-  
Pituitary-Adrenal Axis  
George B. Chrousos, M.D.
- Z01 HD 00619-06 Hypothalamic-Pituitary-Gonadal Interaction  
D. Lynn Loriaux, M.D.
- Z01 HD 00621-05 Mechanism of Linear Growth  
Fernando Cassorla, M.D.
- Z01 HO 00622-05 Diagnostic and Therapeutic Applications of  
Growth Hormone Releasing Hormone  
George R. Merriam, M.D.
- Z01 HD 00623-04 Adrenal Physiology and Pathophysiology  
Gordon B. Cutler, Jr., M.D.
- Z01 HD 00624-01 Cell Membrane Microviscosity  
Richard A. Knazek, M.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987  
Developmental Endocrinology Branch

The Developmental Endocrinology Branch has as its charge the furthering of our understanding of the role of the endocrine system in the complex processes of growth and development.

A corollary of this is to understand the disorders associated with these processes and, where possible, to initiate and evaluate rational therapy for these disorders. The Developmental Endocrinology Branch is dedicated, within this framework, to the concept of clinical investigation. By this, we mean that the broad themes of our research derive from questions that emerge in the setting of the medical management of men, women, and children with disorders referable to these systems. We attempt to confine our attention to research themes that can be approached only by investigators with medical training. These guidelines tend to exclude from study problems that can be addressed by basic scientists in the setting of a university or research institute insuring that we employ the unique resources of the Clinical Center Research Hospital to optimum advantage.

The problems currently under study include topics in reproductive endocrinology in men, women, and children, linear growth and its attendant developmental changes in children, the role of the adrenal gland in development, and its link to homeostasis and the stress response. This summary will not catalogue all of the studies currently under way - they are all listed in the individual reports - but will attempt to highlight emerging concepts, show how these findings alter our current understanding, and how they stimulate new or different lines of investigation for the future.

#### Studies in Reproduction:

Our studies of reproduction in women have taken two general lines: First, we wish to understand the endocrinology of the normal reproductive process, including pregnancy and, second, we wish to gain some control over the process either to enhance fertility or suppress it in a safe, convenient, and reversible way.

Studies directed at understanding the underlying process have yielded some interesting results in the past year.

A central question in human reproduction has been whether or not it has a "seasonal" component. Many lower species and some new world primates are "seasonal" breeders, but this has never been shown for old world primates or man. Earlier work from this branch hinted at seasonal peaks in birth rates in far northern primitive cultures. Overlying social variables, however, were many, and no firm conclusions could be drawn from these tantalizing data. We have now examined this question in an old world primate, the rhesus monkey, using controlled lighting schedules, short vs long days, as an independent variable, and testis size and plasma hormone concentrations as a dependent variable. The results show, conclusively and for the first time, that there is a strong seasonal effect in the reproductive process of old world primates. If this is true for man as well as for the monkey, many interesting implications emerge. For example, evaluations for infertility would need to consider this new variable; times of

optimum and minimum fertility could be predicted, and rational therapy for infertility would need to take this new variable into account. We would like to press our advantage with this finding and attempt to explore the idea in a human population. We need a homogenous population that lives in reasonable harmony with the environment with as little influence from 'high lux' light sources as possible. Our projected course is to begin to evaluate this concept in a selected group of Amish who seem to fit these criteria. Controlling the confounding variables while maintaining necessary rapport will be challenging, but worth the investment if an analog of the rhesus findings can be documented in man.

One of the features of the human reproductive cycle that remains unexplained is the fidelity with which a single egg is ovulated month after month. We have shown, in the last year, that the signal responsible for this phenomenon probably emanates from the ovary itself. The strongest support for this comes from ovulation induction studies in women in which marked changes in the dose or frequency of LHRH administration does not alter the 1 egg - 1 ovulation cycle. The most likely candidate for an ovarian signal informing the pituitary gland that a mature follicle is ready to ovulate is progesterone. We have initiated several studies designed to examine this hypothesis using the potent progesterone antagonist, RU 486, as a probe. We have shown that large doses of RU 486 suppress gonadotropin secretion and can induce luteolysis (doses of 5 mg/kg or greater). We have now examined much lower doses and find that doses of as little as 1 mg/kg alter the reproductive cycle in a manner consistent with an obscuring of a progestin signal from ovary to pituitary gland. Doses of RU 486 as low as this have not been used in any previous studies, and the power of the effect on ovulation is quite unexpected and seemingly can have no other likely explanation. If ongoing studies continue to support these early findings, a fundamental question about the human reproductive cycle will have been clarified, at least in part, and new avenues for the manipulation of fertility will be opened. For example, multiple ovulations theoretically could be induced and regulated in this way. In addition, new possibilities to explain "idiopathic" infertility are suggested - e.g. an abnormal production of the progesterone signal, or its "incomplete" processing' at the pituitary gland.

Essential to the study of reproduction is the study of the endocrine events of early pregnancy. The most powerful maker we have for this process is human chorionic gonadotropic. This molecule is normally made only in the developing syncytiotrophoblast. Recent studies have been directed at better understanding the synthesis, secretion, metabolism and action of this hormone. During pregnancy, not only hCG and its subunits, but also fragments of hCG are excreted into urine. One of these fragments, the beta-core fragment, can constitute as much as 70-80% of the hCG-related molecules evident in urine. Heretofore, little has been known about the metabolic mechanisms for production and urinary excretion of beta-core fragments. To explore this issue, we studied the immunoreactive metabolites derived from infusion of highly purified hCG, hCG-beta, or desialylated hCG in rats. Strikingly, the catabolic products evident in kidney homogenates were essentially identical. In each case, the predominant immunoreactive catabolic products were beta-core fragments. The kinetic behavior of beta-core fragment after formation in the kidney was unusual. Although hCG beta was effectively cleared from the plasma within an hour after injection, the beta-core fragments in kidney had declined only 20% five hours later. These results indicate that the renal parenchymal mechanisms for processing a variety of hCG-related molecules give rise to the same metabolic products, i.e., beta-core fragments. Our finding that beta-core fragments in kidney can be quite long-lived relative to the plasma

kinetics of hCG beta has provided insight into an observation that we made concerning hCG beta metabolism in humans. The day after hCG beta subunit had been cleared from human serum, beta-core fragments were excreted into the urine. The delayed excretion of beta-core fragments relative to hCG beta clearance in humans implied sequestration and processing of the molecule. Our studies in rats clearly identify renal parenchyma as the likely site for these events.

Investigations into hCG-related pregnancy hormones have yielded new insights into their structures and the physiology of glycoprotein metabolism. In normal pregnancy, alpha subunit is secreted both as an uncombined molecule, called free alpha, and as a part of the hCG molecule, wherein it is combined with the beta subunit. Previous findings in this laboratory had demonstrated that the free alpha subunit has more than 2-fold as much sialic acid as the alpha subunit dissociated from intact hCG. These findings were extended in both physiological and biochemical directions in the present period. Chromatography on Ricinagarose, which binds to molecules with terminal galactose residues, and peanut agglutininagarose, which binds to molecules with the disaccharide, gal-gal NAc, revealed that neither free alpha nor dissociated alpha contained terminal galactose residues. Consonant with these structural observations, the *in vivo* plasma clearance rate of free alpha subunit was not significantly different from that of dissociated alpha subunit. Further, unlike desialylated hormones, neither of the subunits was appreciably accumulated by the liver, as would be expected for galactose-terminated glycoproteins. Therefore, though dissimilar in sialic acid content, neither free alpha nor dissociated alpha contains terminal galactose; and, contrary to current dogma, sialic acid content *per se* is not a major determinant of glycoprotein metabolic clearance.

Investigations of the structure-function relationships between native hCG subunits and their proteolytic cores prepared by tryptic proteolysis have produced chemical, physiocochemical, and immunochemical insights. The alpha tryptic core retained full immunopotency but was unable to combine with native beta subunit or, not surprisingly, to interact with LH/CG receptors in rat testis homogenate. The beta tryptic core, on the other hand, combined quite readily with native alpha subunit. It lacked the carboxy terminal peptide (residues 115-145) and contained several cleavage sites within. Nonetheless, except for lacking the carboxy peptide immunodeterminant, it was immunologically intact. The hybrid molecule made by combining native alpha with the tryptic beta core was reduced substantially in affinity for the LH/CG receptor but exhibited full intrinsic activity once bound thereto.

In the current year, this project has contributed advances of both a biochemical and a physiological nature. Our structure-metabolism studies have demonstrated beta-core fragment as the unique metabolic product that is derived from a variety of hCG-related molecules of varying kinetic characteristics. Its apparent universal nature is likely to confer considerable clinical applicability upon beta-core's measurement in patients bearing hCG-secreting tissues. Our research into the structures and functions of naturally occurring forms of hCG alpha subunit have revised certain principles concerning glycoprotein metabolism; that is, the density of sialic acid in glycoproteins without exposed galactose residues is not a major determinant of plasma clearance rate. Knowledge of how carbohydrate structure regulates survival in the plasma and distribution to the tissues is key to the development of therapeutic congeners and to the diagnostic applications of hormone measurement in pregnancy and neoplastic disease.



We have also directed considerable attention to reproductive disorders in men. One of the few treatable causes of infertility in men is that associated with deficient gonadotropin production. The standard therapeutic regimen for this problem was developed in this branch - the combination of parenteral hCG and hFSH. Recent studies by other investigators have suggested that treatment with pulsatile LHRH is superior to this standard regimen, although considerably more labor intensive. We have examined this proposition by studying and comparing the results of both forms of therapy in a large group of men. The findings fail to support the conclusion that pulsatile GHRH is superior to the standard regimen. The only positive advantage seems to be a slightly larger ultimate testis size. Thus, the increased cost and labor of the newer regimen does not seem justified by an improved outcome.

Finally, one of the most serious threats to human health and happiness to appear in recent times, probably since the advent of atomic explosions, is the AIDS epidemic. This is a disease, in large part, tied to the reproductive process. Is there anything peculiar to that process that enhances the infectivity of the AIDS retrovirus? We have been intrigued by the possibility that some property of human semen may play a role as an enhancer of viral infectivity. Our attention was naturally drawn to the prostaglandins because of their great concentration in seminal fluid and their known ability to effect many diverse biologic processes. Since the ability of most cells to interact with their environment is modulated by the fluidity of their cell membranes, we examined the effects of several prostaglandin species on the membrane fluidity of lymphocytes. The results were not unexpected, but surprising in their magnitude. The unique 19-OH prostaglandins of human semen had a profound effect on lymphocyte membranes in a way that would reduce their ability to respond to an infectious challenge. We propose to pursue these findings by studying the infectivity of a series of retroviruses in medium with and without 19-OH prostaglandins. Obvious epidemiologic and preventative medicine implications hinge on these studies.

Short stature, depending on the definition, affects 1% of the population. Most of these children are normal, but some have an abnormality that leads to unfulfilled growth potential. One of the most important challenges facing the endocrinologist is to accurately place these patients into normal or abnormal categories. This requires a better understanding of the stimuli for normal linear growth. In the past year, we have studied the role of three such factors - thyroxine, growth hormone releasing hormone, and somatomedin C (IGF-I).

Using hypothyroid cynomolgus monkeys as a model system, we found that thyroid hormone causes progressive increases in growth velocity over a range of values of plasma thyroxine that lies within the normal range. This suggests that subtle hypothyroidism may be an important and heretofore unrecognized cause of short stature. Derivative clinical studies are in progress to document the veracity and magnitude of this problem in man.

Human growth hormone releasing hormone (HGRH) has been available only for a few years, but in this time we have been able to show that this hormone has great potential as an alternative treatment for children with growth hormone deficiency. An ongoing study suggests that a dose of 1-10mcg/kg/day can restore normal growth velocity in most subjects with growth hormone deficiency. The pattern of administration does not appear to be critical, making a once a day dose schedule feasible. These studies have provided an effective alternative to growth hormone itself, bypassing the issues of Jacob-Kreutzfeld disease associated with



extracted growth hormone and antibody induction associated with the genetically engineered material.

IGF-I has long been believed to be the "mediator" of growth hormone action. This material is now available for testing in primates and man. Studies in growth hormone deficient primates have begun. Obviously, if growth can be induced with this material using a convenient regimen, another potential alternative to growth hormone therapy will be available.

Finally, we have explored the action of sex steroids on the epiphyseal growth plate using direct injections of the steroid into the plate as a means of hormone delivery. The findings of these studies are quite novel and present many possibilities for future work on the mechanism of linear growth. Although the parenteral administration of sex steroids results in a brisk increase in linear growth, the intercartilagenous administration of these hormones is impotent in this regard. These studies suggest that a new and unrecognized family of growth mediators may exist. Studies to exploit these findings are in progress in primates and man.

Two large studies designed to examine new approaches to short stature are in progress. The first explores the effects of suppressing puberty on ultimate stature in short children. The second examines the effects of growth hormone treatment on the ultimate stature of otherwise normal short children. These studies are designed to examine, in a rigorously scientific way, the actual benefit to be derived from these treatments, both of which are being promoted by some members of the pharmaceutical industry in a seemingly self-serving way.

Finally, one of the causes of abnormal stature is the generic disorder of precocious puberty. Our branch has, over the past few years, developed tests to diagnose the various form of precocious puberty and identified the pathophysiologic abnormality underlying the McCune-Albright and familial male forms of the disease. In addition, rational treatments for these disorders have been developed. We are currently evaluating the effects of these regimens on ultimate stature and comparing the results to those of the previously developed LHRH agonist therapy for gonadotropin-dependent central precocity.

The adrenal glands are essential for life and play a central role in growth, development, and the response of the body to stress, both internal and external. How the system is activated by stress and the biologic consequences of this activation are largely unknown. The focus of our studies over the past year has been the physiological role and potential clinical utility of CRF. We have been able to show that stress-mediated activation of the hypothalamic-pituitary-adrenal axis seems to be CRF dependent. This is also true for the pseudo-Cushing's states such as alcoholism, depression, and the psychiatric disorders associated with increased cortisol secretion. Cushing's Disease seems to be CRF independent, suggesting several possible biochemical abnormalities that could occur in the responsible pituitary microadenoma. CRF appears able to differentiate pseudo-Cushing's states from Cushing's disease on the one hand, and Cushing's disease from the ectopic secretion of ACTH on the other. CRF is now the test of choice for the latter distinction. Coupled with inferior petrosal sinus sampling, CRF has allowed the accurate localization of the source of ACTH secretion to the sella so that the surgical cure of this disorder has increased from 50% to better than 95%. This is the most important single advance in the treatment of this disease since the techniques of bilateral adrenalectomy were perfected in the 1940's.

Some cases of ectopic ACTH secretion still cannot be localized on the first evaluation. We have successfully employed RU 486, a potent glucocorticoid antagonist, to temporize in the treatment of this disease until the offending lesion can be identified. Using this approach, several initially inapparent bronchial carcinoids have been found and successfully treated. With these advances, and excepting adrenal cancer, the management of Cushing's syndrome has been largely standardized.

One of the serious problems that remains is the poor treatment options for the pituitary tumors causing Nelson's syndrome. Since we know that these tumors respond to CRF, we have initiated a program directed toward delivering a potentially lethal dose of Boron, an alpha emitter in a neutron flux, to this tumor using CRF as a localizing agent. We have shown that CRF is internalized. Boron has been successfully linked to CRF in carboron cages. We are hopeful that this report next year will contain findings along this line that are as optimistic as those surrounding CRF have been over the past few years.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00613-07 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Clinical and Basic Studies of Male Reproduction

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Sherins

Head

DEB, NICHD

Others: (see attached list)

## COOPERATING UNITS (if any)

(see attached list)

## LAB BRANCH

Developmental Endocrinology Branch

## SECTION

Section on Reproductive Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

8.0

## PROFESSIONAL

7.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

The objectives of this study are to ascertain biological, physiological and clinical mechanisms of male reproductive disorders and to provide rational strategies of treatment for men with reproductive disease.

This project represents a continuum of research begun in 1970 and includes studies of 1) the hormonal regulation of spermatogenesis in gonadotropin deficient men, 2) biology of sperm function, 3) adverse effects of cancer therapy on gonadal function, 4) evaluation of treatment of men with reproductive disorders and 5) the role of sex steroids in regulation of gonadotropin secretion.

Major findings from studies performed this year have shown 1) pulsatile GnRH in gonadotropin deficient men enhances testicular growth above that achieved with gonadotropins but sperm production is not facilitated, 2) subjects with Kallmann's syndrome show evidences for subtle neurological deficits which may serve as markers for the genetic disorder, 3) semen from infertile men show subpopulations of sperm on the basis of linear velocity characteristics, which may be important as a marker of infertility, 4) biodegradable testosterone microspheres appear to provide long-term androgen replacement in hypogonadal men, which offers a potentially practical therapy for men who desire infrequent parenteral injections.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00614-07 DEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Biology of Hormone Binding Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title laboratory and institute affiliation)

PI:	B. C. Nisula	Head	DEB, NICHD
Others:	R. Hiramatsu	Visiting Fellow	DEB, NICHD
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.9

PROFESSIONAL

0.7

OTHER

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

The broad objectives of this project are to understand the transport of hormones to target tissues, the biological functions of binding proteins, and the mechanisms by which they are regulated in human disease. Recent research findings include: Demonstration that a substantial component of blood cortisol is transported within erythrocytes and that the erythrocyte-associated cortisol dissociates extremely rapidly and would therefore be readily available to tissues; and elucidation of new evidence supporting a role for protein-bound plasma hormones, by dint of their bioavailability, in the regulation of hepatic functions. No future studies are planned under this project.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 00615-07 DEB
<b>PERIOD COVERED</b> October 1, 1986 to September 30, 1987		
<b>TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )</b> Steroid Antagonists		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)</b> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>PI: George P. Chrousos</div> <div>Head</div> <div>DEB, NICHD</div> </div> <div style="margin-top: 10px;">Others: (see attached list)</div>		
<b>COOPERATING UNITS (if any)</b> BPB, NIMH (P.W. Gold) SB, NCI (M. Lotze) LCP, CC (T. Fleisher)		
<b>LAB BRANCH</b> Developmental Endocrinology Branch		
<b>SECTION</b> Unit on Hypothalamic Releasing Factors		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS</b> 2.1	<b>PROFESSIONAL</b> 2.0	<b>OTHER</b> 0.1
<b>CHECK APPROPRIATE BOX(ES)</b> <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )</b> <div style="margin-top: 10px;"> <p>Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. <u>Antagonists</u> for the <u>glucocorticoids</u> or the <u>progestins</u> with potential clinical usefulness have been discovered only recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids.</p> <p>Initially, we proved that glucocorticoid antagonists can be developed by modifications of the 11-position of the steroidal C ring of glucocorticoids. Then we tested a prototype <u>glucocorticoid-progestin antagonist (RU 486)</u> developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals.</p> <p>Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that antiglucocorticoids could be used for challenging the <u>hypothalamic-pituitary-adrenal axis</u> when clinical testing is required in patients with disorders of this axis. <u>Antiglucocorticoid therapy</u> of patients with severe <u>Cushing's syndrome</u> due to <u>ectopic ACTH secretion</u> or <u>adrenocortical tumors</u> causes remission of the clinical manifestations of <u>hypercortisolism</u>. We have treated 7 patients and are currently enlarging the therapy series.</p> <p>Given to women in single monthly doses during the luteal phase of the cycle RU 486 causes vaginal bleeding. The subsequent cycle is of normal duration. This suggests that single doses of RU 486 could be used for <u>contraception</u>.</p> </div>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00616-07 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Structure, Function, and Physiology of Glycoprotein Hormones

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI: B. C. Nisula Head DEB, NICHD

Others:	D. Blithe	Sr. Staff Fellow	DEB, NICHD
	S. Rose	Med. Staff Fellow	DEB, NICHD
	R. Wehmann	Special Expert	DEB, NICHD
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD

## COOPERATING UNITS (if any)

LAB/BRANCH Developmental Endocrinology Section

SECTION Medical Endocrinology Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	3.6	PROFESSIONAL	2.8	OTHER	0.8
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## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided )

The general goal of this project is to understand the structure, function, and physiology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances include the following: demonstration of the production of beta-core fragments in kidney during the catabolism of hCG, hCG-beta, or desialylated hCG; elucidation of the structure and kinetic impact of the different oligosaccharide moieties of dissociated alpha and free alpha-subunit of pregnancy; characterization of the structural and functional properties of trypsin-digested subunits of hCG; and identification of hCG as an ovarian follicular growth inhibiting factor. Future directions of the project will include purification of beta-core fragment from pregnancy urine and its characterization with respect to physicochemical, immunological, biological, and physiological properties and the development of a beta-core radioimmunoassay for physiological and clinical studies.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00618-06 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: G.P. Chrousos

Head

DEB, NICHD

Others: (See attached list)

COOPERATING UNITS (if any) BPB, NIMH (P. Gold); CNS Section, SNB, NINCDS (E. Oldfield); LDP, NIMH (E. Sussman, E. Nottelman, G. Inoff); LCP, NIA (M. Blackman, E. Pavlov, M. Harmon); EPL, Dept. of Military Medicine, USUHS (P. Deuster); Dept. of Pediatrics, Temple Univ. (J. Levine-Ross)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Unit on Hypothalamic Releasing Factors

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

8.6

## PROFESSIONAL:

7.1

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic-pituitary-adrenal axis. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas:

- 1) Clinical application of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's syndrome (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, depression and anorexia nervosa.
- 2) Regulation of the hypothalamic-pituitary-adrenal axis in vivo and in vitro: The regulation of the axis by opioids, vasopressin, oxytocin, and glucocorticoids has been studied in vivo. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has been examined in vitro in a newly established hypothalamic organ culture system. Athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents.
- 3) Role and actions of glucocorticoids: The effects of glucocorticoids upon the cardiovascular system during surgical stress are merely permissive. Glucocorticoid resistance is associated with normal size glucocorticoid receptor protein that has decreased affinity for glucocorticoid and normal size mRNA.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00619-06 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Hypothalamic-pituitary-gonadal Interactions

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI:	D.L. Loriaux	Head	DEB, NICHD
Others:	G.R. Merriam	Head, UCN	DEB, NICHD
	L. Nieman	Expert	DEB, NICHD
	B. Albertson	Adjunct Scientist	DEB, NICHD
	P. Manasco	Medical Staff Fellow	DEB, NICHD
	P. Platia	Medical Staff Fellow	DEB, NICHD

## COOPERATING UNITS (if any)

E.E. Baulieu, Roussel-UCLAF, Paris, France, Department of Radiology

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Section on Steroid Hormones

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.63

## PROFESSIONAL

1.63

## OTHER

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

Studies this year have centered on the pathophysiology of Leydig cell activation in patients with familial precocious puberty. This interesting group of patients with familial precocious puberty. This interesting group of patients demonstrate what appears to be gonadotropin independent Leydig cell activity. Previous studies have focussed on the possibility that a humoral factor is responsible for this process. Several groups have searched for this hypothetical factor using in vitro assays of Leydig cell function, usually the dispersed rat Leydig cell assay. These systems have failed to reveal Leydig cell stimulators. We reasoned that the heterologous nature to the assay systems might explain this failure. We examined this hypothesis by infusing plasma from patients with the disorder control group, and from pubertal stage matched controls directly into the spermatic artery of rhesus monkeys. Testosterone secretion into the testicular vein served as the response parameter. This system separated the precocious puberty group from the control group, supporting the hypothesis that a circulating factor may play a role in the pathogenesis of this syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-05 DEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Mechanism of Linear Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Fernando Cassorla

Head

DEB, NICHD

Others: (see attached list)

COOPERATING UNITS (if any)

Clinical Center, NIH (M. Skerda, Gayle Heavener, Janet Jones); Metabolism Branch, NCI (P. Nissley, M. Gelato); Catholic University of Nijmegen, The Netherlands (I.M. Valk); Hahnemann Medical School, Philadelphia, Pennsylvania (J.L. Ross)

LAB BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Linear Growth Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.4

PROFESSIONAL

1.4

OTHER

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include studying the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. In addition, we are studying the mechanism of catch up growth in small for gestational age infants and in a primate model. We are also attempting to define the optimal dose of hydrocortisone for growth in patients with adrenal insufficiency and of thyroid hormone in patients with hypothyroidism. We are also studying the effects of administering somatomedin-C, a growth hormone-dependent peptide, to hypophysectomized cynomolgus monkeys to determine its growth-promoting activity. In addition, we are examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double-blind, placebo-controlled clinical trial. In addition, we are investigating the growth hormone secretory dynamics in patients with hypophosphatemic rickets. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone-deficient children by using different treatment regimens in order to optimize growth.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00622-05 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormone

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: George R. Merriam, M.D.

Head

DEB, NICHD

Others: (see attached)

## COOPERATING UNITS (if any)

University of Catania, Italy; INTR, Chile; Chinese Academy of Medical Sciences, Beijing; University of Minnesota; Dalhousie University

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

NICHD, NIH, Bethesda, MD 20892

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

2.25

## OTHER

0.25

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

Growth hormone-releasing hormone (GHRH) and somatostatin (SRIF) are the two hypothalamic peptides which together control growth hormone (GH) synthesis and release. This project aims: a) to study the neuroendocrine regulation of GH secretion; b) to define alterations in GHRH responses in different physiologic states, and to determine their cause; and c) to explore the efficacy of GHRH and analogues for treatment of GH deficiency and excess. 1) We have studied the mechanism underlying the indirect stimulation of GH by co-administering GHRH with insulin-induced hypoglycemia (ITT). ITT is capable of enhancing the response to even a maximally stimulating dose of GHRH, indicating that ITT must activate mechanisms other than release of endogenous GHRH. The likeliest possibility is that ITT suppresses somatostatin secretion. 2) GH secretion remains pulsatile during the continuous infusion of GHRH in both normal and acromegalic subjects, suggesting that somatostatin secretion is also intermittent, and variably blocks the response to GHRH. The frequency of GH pulses increases during GHRH infusions in normals, but is unchanged in acromegalics. This suggests that SRIF pulsatile secretion can change in response to elevations of GH or GHRH -- a finding we have confirmed in hypothalamic perfusions in vitro -- and that the frequency of episodic secretion in GHRH-infused normals resembles that seen in acromegaly. 3) An ongoing long-term dose-response study suggests that a dose of 10mcg/kg GHRH per day can restore normal growth velocity in many patients with GH deficiency. We do not see a significant difference in response based on the pattern of administration, so long as the total daily dose is sufficient. The enhancement of GH responses to acute doses of GHRH by drugs which alter SRIF secretion has led us to study whether alteration of SRIF might also enhance the growth response to chronic GHRH therapy.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00623-04 DEB
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Adrenal Physiology and Pathophysiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>PI:</div> <div>G. B. Cutler, Jr.</div> <div>Head</div> <div>DEB, NICHD</div> </div> <div style="margin-top: 10px;">       Others: (see attached list)     </div>		
COOPERATING UNITS (if any)  (see attached list)		
LAB/BRANCH Developmental Endocrinology Branch		
SECTION Section on Developmental Endocrinology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS <div style="text-align: right;">4.3</div>	PROFESSIONAL <div style="text-align: right;">4.1</div>	OTHER <div style="text-align: right;">0.2</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div> <input checked="" type="checkbox"/> (a) Human subjects  <input checked="" type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided )  <div style="margin-top: 20px;"> <p>We seek to advance understanding of the mechanisms that cause <u>adrenal androgen</u> secretion by the <u>fetal adrenal zone</u> prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as <u>premature adrenarche</u>, <u>congenital adrenal hyperplasia</u>, <u>adrenal neoplasma</u>, <u>idiopathic hirsutism</u>, <u>polycystic ovary syndrome</u>, and <u>Cushing's syndrome</u>. We also seek to clarify the pathophysiology of <u>primary adrenal insufficiency (Addison's disease)</u> and <u>secondary adrenal insufficiency</u> and to improve the treatment of these conditions.</p> </div>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00624-01 DEB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Cell Membrane Microviscosity

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	R. Knazek	Head	DEB, NICHD
Others:	P. Feuillan	Adjunct Scientists	DEB, NICHD
	R. Whitcomb	Medical Staff Fellow	DEB, NICHD
	D. Liu	Summer Student	DEB, NICHD
	T. Garvey	Summer Student	DEB, NICHD
	I. Losconczy	Adjunct Scientist	DEB, NICHD
	Y. Wu	Adjunct Scientist	DEB, NICHD
	R. Das	Adjunct Scientist	DEB, NICHD

## COOPERATING UNITS (if any)

Surgery Branch, NCI, NIH, Lab Microbiology and  
Immunology, NIDR, NIH

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Unit on Cell Membrane Physiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

## TOTAL MAN-YEARS

4.5

## PROFESSIONAL

3.5

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

Changes in the microviscosity of cell membranes were shown to modulate the functionality of their receptors, presumably by altering their ability to reorient into more or less active positions. 1) The abnormal accumulation of saturated long chain fatty acids in the inherited disease of adrenoleukodystrophy also result in stiff membranes for when human adrenocortical cells are cultured in their presence, 15 times more ACTH is needed to achieve a given level of cortisol synthesis. This probably explains the mechanism by which adrenal insufficiency occurs in this disease. 2) Gossypol is a dietary phenolic that has caused outbreaks of infertility in several provinces in China. This compound causes an increase in granulosa cell membrane microviscosity and thereby decreases their estrogen response to FSH. Altered functionality of gonadotropin receptors on target tissues is a likely mechanism by which this phenomenon of infertility occurs. 3) We have shown that the unique 19OH prostaglandins in human semen suppress lymphocytes' response to mitogens 30 times more than PGE<sub>2</sub>. Preliminary studies demonstrated that they reduce the microviscosity of human lymphocyte membranes, a phenomenon that may be extremely important in explaining the increased susceptibility of homosexuals to various infective agents. Other preliminary studies have shown that these same prostaglandins cause a marked decrease in the microviscosity of human sperm membranes, an observation that may be relevant to their role in male reproductive physiology. A direct link between the immune surveillance system and the adrenal gland was established when human monocytes were shown to elaborate a soluble factor that stimulates human adrenocortical cells to synthesize cortisol *in vitro*. Preliminary studies have indicated that the monocytes and granulocytes of diabetic patients metabolize arachidonic acid in an abnormal fashion. The identity of these metabolites is being established and the possible role in the microvascular disease of diabetes is being studied.



## ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH

- Z01 HD 00022-14 Renin-Angiotensin System and Aldosterone Regulation  
Greti Aguilera, M.D.
- Z01 HD 00035-15 The Structure and Function of Biologically Active Molecules  
Hao-Chia Chen, Ph.D.
- Z01 HD 00146-12 Structure and Function of Chorionic Gonadotropins  
Hao-Chia Chen, Ph.D.
- Z01 HD 00147-12 Mechanism of Action of Peptide Hormones in Steroidogenic Cells  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00149-12 Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00150-12 Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00151-12 Regulation of Gonadal and Placental Function  
Kevin J. Catt, M.D., Ph.D.
- Z01 HD 00184-09 Regulation of Pituitary Hormone Secretion  
Kevin J. Catt, M.D., Ph.D.
- Z01 HD 00187-08 Hormonal Regulation of Cellular Metabolism  
Kuo Ping Huang, Ph.D.
- Z01 HD 00190-05 Adrenocortical Zonation: Regulation of Steroidogenesis and Cholesterol Metabolism  
Charles A. Strott, M.D.
- Z01 HD 00191-03 Mechanisms of Neuroendocrine Regulation  
Greti Aguilera, M.D.
- Z01 HD 00192-02 Purification, Immunology, and Functional Activity of Adrenocortical Proteins  
Charles A. Strott, M.D.
- Z01 HD 00193-02 Angiotensin II Receptors and Activation Mechanisms  
Kevin J. Catt, M.D., Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Endocrinology and Reproduction Research Branch

The research programs of the Endocrinology and Reproduction Research Branch are directed at the elucidation of cellular mechanisms involved in hormone secretion and action. These programs include studies on the characterization of peptide hormones and their cellular receptors; the structure-function relationships of peptide and glycoprotein hormones; the regulation of hormone biosynthesis and secretion; and the mechanisms of peptide hormone action in endocrine target cells. Of particular interest are the analysis of pituitary-gonadal and pituitary-adrenal regulation, the control of ovarian activity during the reproductive cycle and pregnancy, and the receptor-mediated control of pituitary, gonadal, and adrenal function. During the current year, research has been performed on the receptors and signalling processes that are responsible for the control of steroid production in endocrine target cells. The role of hormones in cellular regulation has also been examined in selected forms of normal and disordered human endocrine function, and in appropriate animal model systems for the analysis of hormone secretion and the stimulatory and inhibitory control of target-cell function. The staff of the ERRB share common interests in the mechanisms of action of peptide and glycoprotein hormones, the role of neuropeptides in hypothalamic-pituitary regulation, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system and aldosterone secretion, and the role of protein phosphorylation in metabolic regulation. The major research programs of the Branch are supervised by the respective senior investigators under the following organizational units within the ERRB.

(a). The Section on Hormonal Regulation. (Dr. Kevin Catt) performs research on the control of endocrine target cells by peptide hormones, in particular the characterization, regulation, and activation mechanisms of membrane receptors for gonadotropin-releasing hormone (GnRH), corticotropin-releasing factor (CRF), angiotensin II, and gonadotropins. The receptor-mediated actions of hypothalamic releasing peptides and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells. The actions of angiotensin II and gonadotropins are studied in rat or bovine adrenal glomerulosa cells, and ovarian granulosa or luteal cells, respectively.

The hypothalamic control of reproductive function is expressed through the actions of GnRH, which regulates gonadotroph function and LH secretion by binding to high affinity receptors in the plasma membrane. GnRH receptors appear to be confined to the pituitary and placenta in primates, but are present in gonads, brain, and other sites in the rat. The mechanism of GnRH receptor activation in gonadotrophs has been shown to involve the integrated actions of several intracellular messenger systems. These include phosphoinositide breakdown and mobilization of intracel-

lular calcium, as well as the influx of extracellular calcium. In isolated gonadotrophs, GnRH stimulates the hydrolysis of phosphatidylinositol bisphosphate to diacylglycerol and inositol trisphosphate ( $\text{InsP}_3$ ). A role for diacylglycerol and activation of protein kinase C in gonadotrophs has been suggested by studies on the translocation of protein kinase C and its regulation by activators (phorbol esters, synthetic diglycerides) and inhibitors (retinal). Also, the generation of  $\text{IP}_3$  and promotion of calcium mobilization and entry provides a mechanism for the early elevation of  $[\text{Ca}^{2+}]_i$  during GnRH action. GnRH was found to stimulate the production of several higher inositol phosphates ( $\text{IP}_3$ ,  $\text{IP}_4$ ,  $\text{IP}_5$ ) and to cause marked elevation of  $\text{Ins-4-P}$  rather than  $\text{Ins-1-P}$  as the major product of polyphosphoinositide metabolism. Arachidonic acid (AA) and its lipoxxygenated metabolites also mediate GnRH action, and are generated via activation of diacylglycerol lipase as well as phospholipase A2. The actions of AA on LH release are related to its effects on calcium mobilization and activation of an AA-dependent protein kinase in pituitary cytosol. The role of calcium entry in GnRH action has been shown to be related to the time course of the LH response, which is at first independent of extracellular calcium but is subsequently dependent on calcium influx during the sustained phase of LH release in GnRH-stimulated gonadotrophs.

The properties of angiotensin II (AII) receptors and their intracellular signalling pathways were studied in the adrenal zona glomerulosa and other target tissues. The mechanisms leading to stimulation of steroidogenesis were analyzed in isolated glomerulosa cells from the rat and bovine adrenal cortex. Purification of photoaffinity labeled AII receptors of the bovine adrenal gland was pursued by detergent solubilization and fractionation by ion exchange and lectin-affinity, and immunoaffinity chromatography. Elevation of cytoplasmic calcium by AII depends upon mobilization of intracellular calcium stores by the products of ligand-stimulated phosphoinositide turnover, and also on calcium entry through voltage-sensitive channels. Microsomal receptors for inositol-1,4,5-trisphosphate, previously identified in adrenal microsomes, were also demonstrated in the anterior pituitary gland. The  $\text{Ins-1,4,5-P}_3$  formed from  $\text{PIP}_2$  breakdown during AII action was rapidly eliminated via two metabolic routes. In addition to breakdown via  $\text{Ins-1,4-P}_2$  and  $\text{Ins-4-P}$  via the previously identified 4-monophosphate pathway, the calcium mobilizing 1,4,5-trisphosphate is rapidly converted to  $\text{Ins-1,3,4,5-P}_4$ , which is then degraded to the inactive 1,3,4-trisphosphate isomer. The latter is metabolized by degradation to  $\text{Ins-3,4-P}_2$  and  $\text{Ins-1,3-P}_2$ , and also by a further cycle of phosphorylation to form a novel tetrakisphosphate isomer, recently identified as  $\text{Ins-1,3,4,6-P}_4$ . These studies have further indicated the importance of the 4-monophosphate pathway in inositol polyphosphate catabolism, and have revealed new phosphorylation pathways and inositol metabolites with potential roles in intracellular signalling and AII action in the glomerulosa cell and other target tissues.

The molecular basis of hormone action during cellular differentiation and growth was studied using the ovarian granulosa cell as a model system, with emphasis on the functions and mechanisms of action of growth factors and gonadotropins during granulosa cell development. Although growth factors alter the proliferation of a wide variety of epithelial cells, they have negligible mitogenic effects in the rat granulosa cell. Rather, their actions are expressed on the differentiative potential of ovarian cells. Studies with the TGF- $\beta$  have demonstrated bifunctional actions of this growth factor on the maturation of granulosa cells. The stimulation of cAMP formation, steroidogenesis, and LH receptor expression by FSH in cultured granulosa cells was altered by TGF- $\beta$  in a concentration-dependent manner. In the presence of suboptimal amounts of FSH, TGF- $\beta$  amplified gonadotropin responses. However, as the levels of FSH were elevated, TGF- $\beta$  had less effects or even inhibited FSH action in granulosa cells. The inhibitory effects of TGF- $\beta$  were observed only in the presence of insulin, suggesting that the total complement of hormones and growth factors within ovarian follicles determine the eventual development of granulosa cells. Further, TGF- $\beta$  was shown to interact during granulosa cell development with an additional growth factor, EGF, by directly modulating EGF receptors. FSH increased EGF receptor number during granulosa cell differentiation through its elevations of cAMP levels. TGF- $\beta$  augmented the effects of FSH on EGF receptors, as well as increasing these binding sites in the absence of gonadotropin. The effects of TGF- $\beta$  alone on EGF receptors are somewhat unique in that an overwhelming majority of demonstrable effects of growth factors in the ovary involve modulation of gonadotropin action. Thus, growth factors can directly modify ovarian function, as well as having a permissive role in the biological responses induced by FSH. The enhancement of EGF receptors by TGF- $\beta$  resulted in a parallel rise in the inhibitory effects of EGF on FSH-induced cAMP production and LH receptor formation. An analysis of other antagonistic or cooperative effects of growth factors in the ovary may reveal information relevant to the control of reproductive function.

In studies on the expression of specific proteins induced by FSH in granulosa cells, gonadotropin was shown to stimulate the production of a tissue-type plasminogen activator that is localized to the cell-surface. In addition, both FSH-treated and control cells synthesize intracellular urokinase-plasminogen activators. In contrast to many other cells that synthesize and rapidly secrete plasminogen activators, these enzymes remain associated with granulosa cells throughout the differentiation process. These studies are noteworthy in that the elevation of plasminogen activator by FSH is one of the earliest reported biological responses of gonadotropin in granulosa cells. The majority of actions induced by FSH, such as steroid production and formation of receptors for LH and prolactin, are not expressed until 24 hours or more of culture. Thus, analysis of plasminogen activator biosynthesis may allow an assessment of regulatory pathways of hormone action during the early differentiation stages of granulosa cells. The production of specific plasminogen



activators was assessed through immunological methods and antibodies to tissue-type plasminogen activator fully neutralized plasminogen activator responses in intact granulosa cells, suggesting the presence of a cell-surface enzyme. Localization of plasminogen activator at the cell-surface suggests that the enzyme may be directly involved in remodeling responses of these cells as they interact with the extracellular matrix during their migration and aggregation into functional, steroid-secreting complexes.

The Section on Endocrine Physiology. (Dr. Greti Aguilera) investigates physiological and pathological aspects of circulatory homeostasis and neuroendocrine regulation, including mechanisms of adaptation to stress. Part of the program is focused on the role of the renin-angiotensin system in the regulation of mineralocorticoid secretion and blood pressure, and has been extended to effects of AII in other systems such as pituitary and gonadal function. AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa zone to AII. Previous studies in the rat have demonstrated that the adrenal responsiveness to AII depends on the trophic effects of the peptide and the modulatory effects of other regulators such as dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF). Studies on the adrenal effects of SRIF were extended to the primate. SRIF-like immunoactivity was identified in monkey and human adrenal cortex, and the peptide was found to preferentially inhibit AII-stimulated aldosterone production in isolated monkey adrenal cells. These studies support a role for SRIF in the control of aldosterone secretion in primates. Studies on the mechanisms of action of aldosterone regulators were focused on the effects ANF in the adrenal and pituitary. ANF was a potent stimulatory of cGMP production, but the cyclic nucleotide had no inhibitory effect on steroidogenesis. ANF inhibited aldosterone secretion without affecting AII-induced increases in cytosolic calcium or phospholipid turnover. Incubation of adrenal cells with arachidonic acid or induction of its release by phospholipase A<sub>2</sub> or melittin inhibited aldosterone secretion with characteristics similar to those of ANF. These studies have demonstrated that the inhibitory effect of ANF on aldosterone production is not mediated by cGMP, and suggest that arachidonate metabolism may have a role in this regard. In rat pituitary cells, ANF was a potent stimulatory of cGMP production but had no effect on basal or stimulated hormone production. In the gonads, AII receptors were found to be present in interstitial cells in the testes and granulosa and luteal cells in the ovary of rats and primates. In the ovary, AII receptor activation is coupled to increases in cytosolic calcium. The presence of AII receptors in the testes and ovaries suggest a role for the peptide in the regulation of gonadal function.

Studies on neuroendocrine regulation have focused on the regulation and actions of corticotropin releasing factor (CRF) receptors, and the interactions of CRF with other ACTH regulators. Previous studies revealed that pituitary receptor down-regulation



and desensitization that accompanies the increase in plasma ACTH after adrenalectomy is partially due to increased hypothalamic CRF secretion. Studies in rats with hypothalamic lesions demonstrated that the effect of adrenalectomy is completely dependent on hypothalamic factors. Glucocorticoid deficiency per se is not involved since physiological amounts of corticosterone decrease CRF receptors. Desensitization of plasma ACTH responses during prolonged stress is accompanied by CRF receptor down-regulation and desensitization of cAMP responses to CRF. However, pituitary responsiveness in vivo is maintained or increased, probably due to the presence of spare/CRF receptors and other regulators. In cultured pituitary cells from chronically stressed rats, cyclic AMP and ACTH responses to CRF were reduced but the responses were recovered by simultaneous incubation with vasopressin. Brain CRF receptors were unchanged during glucocorticoid administration and stress. These studies have demonstrated that CRF receptor desensitization is not reflected in decreased corticotroph responsiveness, and emphasize the importance of the interactions between CRF and other regulators during physiological regulation of ACTH release.

Previous studies on the mechanism of action of ACTH regulators have shown that the effect of CRF is cAMP-dependent, while other stimuli increase ACTH secretion and potentiate the stimulation by CRF through calcium/phospholipid-dependent mechanisms, with activation of protein kinase C. Further studies have demonstrated that potentiation of CRF action by VP involves enhancement of CRF-stimulated cAMP levels by two mechanisms: inhibition of phosphodiesterase, and protein kinase C dependent phosphorylation of a component of adenylate cyclase. Regulation of corticotroph function also involves dual effects of arachidonic acid metabolites, with lipooxygenase and cyclooxygenase products being stimulatory and inhibitory, respectively. Kinetic studies in cultured pituitary cells showed two phases of ACTH secretion by CRF and cAMP-independent stimuli: an early phase with a rapid increase in ACTH release rate which is independent of extracellular calcium, and a late phase with a constant secretion rate with partial calcium dependence for CRF, and complete calcium dependence for non-cAMP dependent stimuli.

(b). The Section on Molecular Endocrinology. (Dr. Maria Dufau) investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. A major aspect of this program is concerned with the characterization of gonadal gonadotropin and prolactin receptors, and of the physical and functional relationships of the LH receptor site and adenylate cyclase.

In previous years, the LH/hCG receptor was isolated from the luteinized rat ovary and identified as a single protein (Mr=75,000) on SDS-PAGE by silver staining. The homogeneity of the purified receptor was recently confirmed by microsequencing. Autoradiographic analysis of SDS-PAGE of labeled hCG (with label

only in  $\alpha$ -subunit) crosslinked to pure receptor showed two radioactive bands of  $M_r = 134,000$  and  $97,000$  which corresponded to the receptor hCG  $\alpha\beta$  complex and  $\alpha$ -subunit, respectively. This was confirmed using cross-linked hCG subunits instead of native labeled hCG. Taking into account the contribution of hCG or  $\alpha$ -subunit, the  $M_r$  of the receptor was calculated to be  $79,000$ . Cross-linking studies performed after binding reconstituted hCG (radiolabeled in the individual subunits) to the purified LH/hCG receptor indicated that the hCG  $\alpha$ -subunit predominantly interacts with the receptor molecule. The influence of the  $\beta$ -subunit in this interaction seems to occur mainly through its association with the  $\alpha$ -subunit, presumably by conferring specificity to the  $\alpha$ -subunit for its interaction with the receptor. The  $\alpha$ -subunit, which is identical within species, has an important role in the receptor binding interaction and biological activity of glycoprotein hormone. Characterization of the purified radioiodinated receptor by SDS-PAGE and autoradiography showed a single band of  $M_r = 78,000$ .

These values were consistent with those obtained for protein bands from unlabelled receptors and by cross-linking of hormone-receptor complex. Treatment of labelled receptor with glycosidases demonstrated that the receptor molecule is predominantly N-linked glycosylated by N-linked sugars. Comparison of  $M_r$  values derived from SDS gels with those from fast performance liquid chromatography suggested that the native LH holoreceptor exists in a dimeric form. The labelled hormone was bound by non-denatured blotted monomeric and dimeric forms of the receptor. The Leydig cell receptor was also purified to homogeneity and shown to be of  $M_r 90,000$ , and appeared to exist associated in dimers of identical subunits. The pure receptor can be phosphorylated in vitro by catalytic subunit of cAMP-dependent protein kinase ( $\sim 0.3$  mol of phosphate per mol of receptor). This phosphorylation did not affect the binding characteristics of the receptor. It is likely that receptor dimerization and possible further aggregation are necessary for signal transduction, and receptor phosphorylation by one or more kinases may be involved in regulating gonadotropin action.

The first in vitro LH bioassay term RICT (rat interstitial cell testosterone) for measurement of circulating LH in human and animal species, 5-fold more sensitive than conventional radioimmunoassay, was developed by Dr. Dufau and associates in 1975. During the last three years, development of a simplified and rapid method of comparable sensitivity for the measurement of LH/hCG in plasma, serum, biological fluids, tissue extracts or incubation media was accomplished. The bioassay uses microtiter plates and the product, measured by an enzyme-linked immunoassay procedure using transfer solid phase. The procedure can be carried out in less than five hours (versus  $> 24$  hr for RICT) with minimal reagent preparation. The solid-phase reagent can be added to the reaction tube during or after the biological amplification and without transfer of samples from one set of wells to another. The principle and steps presented can be used also for the assay of

pituitary and hypothalamic hormones or any protein hormone that can stimulate the release of a cell product which can be measured by the present approach (ie. steroid, cyclic nucleotide, gonadotropin).

The biological activity of LH secreted in response to endogenous and low-dose exogenous GnRH pulses in normal men was analyzed by RICT assay. The absence of non-specific plasma effects in the LH bioassay was demonstrated by the finding of undetectable levels of LH bioactivity despite low but measurable immunoactivity in 10 hypogonadotropic men. In normal men, low-dose (10 µg) iv GnRH administration resulted in preferential release, in a pulse-like fashion, of bioactive LH with a significant increase in the median plasma bio- to immunoactive LH ratio. Further pulsations of similar characteristics could be observed with intravenous pulse-like administration of GnRH (10 µg), every 2 hours. This pattern mimicked that of endogenous LH pulsatility. Such increases were not evident in previous studies of men and postmenopausal women in response to exogenous LHRH using either a single large bolus of LHRH (100 µg subcutaneously) or continuous infusion with low doses of LHRH (2 µg/min). This could be explained in the former case by an increased secretion from all pituitary pools yielding an integrated B:I value instead of selective increases from the highly bioactive pool, and in the latter by a lack of definition of a small early pool (presumably of high bioactivity) and considerable mixing with the late pool of reduced bioactivity. The preferential increase in plasma bioactive LH in response to exogenous or endogenous GnRH might reflect in the smaller initial distribution volume and slower metabolic clearance rate of bio versus to immuno LH. Other studies have demonstrated a sustained inhibitory actions on Bio and immunoactive LH<sub>3</sub> of a potent antagonist of GnRH (N-acetyl-D-pCl-Phe<sup>1,2</sup>-D-Trp<sup>3</sup>-D-Ala<sup>10</sup>GnRH<sup>10</sup>) in postmenopausal women. The GnRH antagonist binds avidly to serum proteins and has a prolonged plasma residence time, which may explain its extended duration of action.

Stimulation of the androgen pathway occurs mainly through cAMP-mediated mechanisms, and can be negatively influenced by the action of certain hormones. Recent studies demonstrated the presence of functional angiotensin II (AII) receptors in the rat Leydig cell, with high affinity ( $K_d=1.7$  nM) and low capacity (2,000 sites per cell). AII inhibits GTP-and LH-stimulated adenylate cyclase in Leydig cell membranes. This hormone also acutely inhibits LH stimulation of cyclic AMP pools and testosterone production in Leydig cells. These effects were prevented by incubation with pertussis toxin and reversed by 8-bromo cAMP additions, indicating that AII action in the Leydig cell occurs through the guanyl nucleotide inhibitory unit of adenylate cyclase (Gi), findings that have further emphasized the predominant importance of the cAMP pathway in the Leydig cell. A number of studies have provided evidence for the presence of renin-angiotensin system in reproductive tissues. Recent studies from the Section have demonstrated that angiotensin-converting enzyme activity in rat testis is localized predominantly in the germinal



cells with only minor<sub>3</sub> activity in purified adult Leydig and Sertoli cells. Also, [<sup>3</sup>H]captopril bound specifically to cellular fractions enriched in germinal cells. Because of the predominant localization of angiotensin-converting enzyme in the testicular tubular elements, it is likely that AII exerts a physiological paracrine regulatory function. The locally produced hormone could exert homologous negative modulatory influence on hormonal-stimulated events in the Leydig cells.

In addition to the potentiation a direct effect of forskolin, inhibitory effects of low-dose (pM) forskolin on basal and hCG-stimulated cyclic AMP pools and testosterone production were observed, and were shown to involve inhibition of adenylate cyclase via direct or indirect activation of Gi. The finding of such a high-affinity inhibitory action of forskolin is of general value for direct evaluation of functional Gi activity.

Analysis of the mechanisms responsible for induction of early and late steroidogenic lesion was continued. Unlike the adult Leydig cell, the fetal and immature cell is refractory to desensitization and maintains up-regulated LH receptor and steroidogenic functions. Their resistance to desensitization by gonadotropin is attributed to the absence of estrogen-mediated regulation of the androgen pathway, due to low aromatase activity, undetectable E<sub>2</sub> production and low E<sub>2</sub> receptors. The fetal testis contained a predominant fetal cell population (90%) and a small population of transitional cells (5%) with morphological characteristics of cells found in 15 day post-natal testis but with functional capacities of the adult cell. Fetal Leydig cells can be maintained in culture for extended periods with upregulated function by low doses of gonadotropin each 3 days. Treatment of fetal cultures with estrogen caused increase of E<sub>2</sub> receptors and nuclear actions; high- or frequent-dose-gonadotropin treatment increased aromatase activity to levels necessary for induction of desensitization. These studies have demonstrated the emergence of a functional adult-like cell type from the fetal Leydig cell population. The cultured fetal Leydig cell system provides a useful model to elucidate LH and GnRH action,  $\beta$ -endorphin regulation, and the mechanisms involved in the development of gonadotropin-induced estradiol-mediated desensitization of steroidogenesis. Future research with this system will help to further clarify the modulatory mechanisms responsible for the emergence of the adult Leydig cell population.

(c). The Section on Adrenal Cell Biology. (Dr. C. Strott) investigates the physiology and regulation of adrenal steroidogenesis, by characterization of cellular steroid binding proteins and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. The Section is currently interested in the development of adrenocortical zonation and the regulation of adrenal steroidogenesis, and is currently concentrating on two areas of research: 1) adrenocortical calmodulin, calcium- and calmodulin-binding proteins, protein kinase systems, and the



post-translational modification of proteins; 2) purification, immunology, and functional activity of soluble and membranous adrenocortical proteins including steroid-binding proteins.

The mechanism of action of ACTH is only partially understood. Adrenal steroid production is rapidly activated and deactivated ( $\sim 2$  min); such a process is considered too rapid to involve regulation at the level of translation. Regulation would, however, be compatible with protein modification. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone by a cytochrome P450 enzyme system (P450<sub>SCC</sub>). There is as yet no evidence that P450<sub>SCC</sub> is subject to modulation by a phosphorylation-dephosphorylation mechanism. ACTH is known to activate systems which increase the availability of cholesterol for steroid production, but although the latter systems are known to be modulated by phosphorylation-dephosphorylation mechanisms, none is known to be regulatory. There is a complete lack of understanding as to what mechanisms are involved in the intracellular shuttling of cholesterol (substrate) and pregnenolone (product) - compounds which are very water insoluble - in a system where the steroidogenic enzymes are compartmentalized.

The guinea pig provides a valuable animal model to explore the responsivity of different zones of the adrenal cortex to ACTH. In this cortisol-secreting species, ACTH does not stimulate cholesterol side-chain cleavage activity and steroid production in the inner zone, in contrast to the outer zone. Adenylate cyclase activation and cAMP formation are similar for the two zones. Over the past year several interesting observations have been made in the ongoing examination of this model. In the area of cholesterol availability, HMG-CoA reductase (rate-limiting in cholesterol synthesis) was increased in both the outer and inner zones by ACTH. This finding is in contrast to previously reported changes in the LDL receptor (supplies cholesterol derived from extracellular sources), which was stimulated by ACTH in the outer zone but not in the inner zone. When animals were given dexamethasone to suppress endogenous ACTH, HMG-CoA reductase activity was reduced only in the outer zone. Evidence was obtained that ACTH altered the phosphorylation-dephosphorylation status of HMG-CoA reductase in the two zones in a strikingly different manner. This will require careful analysis, because regulation of HMG-CoA reductase activity involves, in part, a kinase, a kinase kinase, and phosphatases. The adrenocortical LDL receptor is also phosphorylated by a specific kinase, but this has not yet been examined in the guinea pig model.

It was found that soluble cAMP-dependent,  $\text{Ca}^{2+}$ /phospholipid-dependent, and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase activities were significantly higher in the outer zone than in the inner zone, by 70%, 60%, and 800%, respectively. In contrast, membrane kinase activity for the three systems demonstrated essentially no zonal difference. Although the physiological meaning of a zonal difference in soluble protein kinase activity is not yet clear, the marked difference in  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase

activity between the outer and inner zones correlates well with the marked difference in steroidogenesis that exists between the two zones. Of the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinases known to exist, there is preliminary evidence to suggest the presence of kinase III in the guinea pig adrenal cortex. The exact nature of the  $\text{Ca}^{2+}$ /calmodulin-regulated kinase(s), however, must await further purification and a more complete characterization. Gel profiles of protein phosphorylation induced by the three kinase systems revealed distinct qualitative and quantitative differences between the outer and inner zones (both soluble and membranous preparations). Since individual phosphoproteins are difficult to identify, gel profiles must be considered preliminary findings.

In addition to purifying  $\text{Ca}^{2+}$ /calmodulin-dependent kinase III, a major effort will be made to isolate an endogenous substrate for kinase III, a  $M_r$  100,000 protein which recent evidence suggests is elongation factor-2 (EF-2). EF-2 is a soluble protein which plays an indispensable role in protein synthesis by promoting the translocation of the new peptidyl-tRNA in the A-site to the P-site as the ribosome moves three nucleotides along the mRNA molecule. EF-2 is a GTP-binding protein that undergoes ADP-ribosylation (inactivation), and is, thus, another member of the growing list of "G-proteins." In the guinea pig adrenocortical model, data have been obtained which indicate phosphorylation of a 100 kD soluble protein by a  $\text{Ca}^{2+}$ /calmodulin-dependent kinase (kinase III); the same protein appears to also undergo ADP-ribosylation. This is an extremely interesting finding, and may reveal a new aspect of adrenal regulation.

It was found that exogenous calmodulin was phosphorylated on threonine residues; this was particularly noteworthy for the inner zone. Phosphorylation of calmodulin appeared to decrease as the concentration of  $\text{Ca}^{2+}$  was increased. The kinase that phosphorylates calmodulin is currently under investigation, and calmodulin-binding proteins are being examined. In cytosol fractions, 56 kD and 47 kD bands have been identified and are clearly more prominent in the outer zone. In microsomal and mitochondrial fractions, 120 kD and 140 kD bands are very prominent in the outer zone but quite faint in the inner zone. In fact, 56 kD and 47 kD calmodulin-binding proteins are present in soluble and membranous fractions while 120 kD and 140 kD calmodulin-binding proteins appear to be present only in the membranous fractions and are much more prominent in the outer zone than in the inner zone.

Finally, the guinea pig adrenocortical model contains specific soluble proteins which bind cholesterol and pregnenolone, the substrate and product of the rate-limiting step in steroidogenesis. The pregnenolone-binding protein has a MW of 58,000 determined by gel permeation chromatography and 34,000 by SDS-gel electrophoresis. Antibodies raised against the 34 kD protein immunoprecipitates pregnenolone-binding activity from cytosol. When antibody-bound protein is eluted from a protein A-IGG column, a 34 kD protein is generated. Antibodies raised against a co-purifying 29 kD protein do not interact with pregnenolone-binding

activity. In addition to the non-catalytic steroid-binding proteins, efforts are underway to isolate and produce antibodies to the P450<sub>scc</sub>. These antibodies will be used to quantitate the steroid-binding proteins and P450<sub>scc</sub> in the outer and inner zones and to examine their intracellular location under different physiological conditions. Antibodies will also be used to isolate specific mRNA for determination of quantitative responses to various manipulations as well as developing cDNA probes. Why does the inner adrenocortical zone fail to respond to ACTH in the same way as the outer zone? The only known function of the adrenal cortex is to produce steroid hormones. Inner zone cells are derived from outer zone cells (probable but not proven) and increase in number with the age of the animal. Thus, the inner adrenocortical zone (zona reticularis) appears to be related in some way to aging of the adrenal cortex.

(d). The Section on Molecular Structure and Protein Chemistry. (Dr. H.C. Chen) conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides and proteins. This includes the identification and synthesis of unusual structures and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis. Of particular interest are the structural design, chemical synthesis, and modification of molecules important to reproductive and developmental biology.

In the current year, broad spectrum anti-microbial substances (Magainins) isolated from skin of Xenopus laevis have been purified to homogeneity by reverse-phase high performance liquid chromatography. Amino acid sequence analyses by Edman degradation and carboxypeptidase Y digestion have revealed two sequences, magainins I and II, which differ in two substitutions shown in parentheses for II: G-I-G-K-F-L-H-S-A-G(K)-K-F-G-K-A-F-V-G-E-I-M-K(N)-S. The sequences have been confirmed by both chemical synthesis and cDNA sequence analyses. Circular dichroism studies indicate that magainin peptides do not form  $\alpha$ -helices in aqueous buffer. However, magainin I and II display 24% and 26%  $\alpha$ -helical conformation in lipophilic solution (40% trifluoroethanol), respectively. These results indicate that the peptides are amphiphilic in structure, and suggest that the lytic action of magainins may stem from highly selective penetration into bilayers of microorganisms. Syntheses of analogues, with emphasis on the substitution of amino acid residues to enhance  $\alpha$ -helix formation and to identify structural requirements for agonist activity, are being pursued. Furthermore, the availability of active synthetic magainin peptides should facilitate studies on the mode of action and potential clinical application in combatting a wide variety of infection.

A unique peptide comprising the last 37 residues of human chorionic gonadotropin  $\beta$ -subunit, which is not found in other glycoprotein hormones, has been synthesized and purified. Through the thiol function of Cys<sup>2</sup>, the peptide is coupled to m-maleimidobenzoylated  $\epsilon$ -amino groups of Keyhole Limpet hemocyanin.



This two-step conjugation approach yields a conjugate which contains 2:1 of peptide to protein ratio (by weight) and permits the maximal exposure of antigenic sites. Antibodies against the conjugate were raised in rabbits and are now in use for a highly hCG-specific and sensitive immunoradiometric assay. The assay can monitor low levels of hCG and hCG-like substances in tissues and biological fluids, and should be important in the determination of early fetal loss and the diagnosis of trophoblastic neoplasms. Studies on the structure and function of chorionic gonadotropin have focused on the role of carbohydrate structures of hCG for the association-dissociation of subunits as studied by the fluorescence enhancement of 1-anilidonaphthyl-8-sulfonate when it binds to the  $\alpha$ -subunit associated molecule. Kinetic data indicate that the carbohydrate moieties are not essential for the association of subunits but influence the rate of association. The association begins with a second-order rate as the rate-limiting step, followed by the first order conformation transition of the  $\alpha\beta$  complex. The influence of carbohydrate appears to reside at the first step and the carbohydrate moieties in the  $\alpha$ -subunit play the dominant role. Pregnant mare serum gonadotropin (PMSG) has been purified and characterized. Since PMSG possesses both LH and FSH activities in rodent, it is important to investigate the role of carbohydrate in the gonadotropic action. In the area of glycoprotein chemistry, a sensitive and reliable method of monosaccharide analysis by a reverse-phase high performance liquid chromatography has been developed.

(e). The Section on Metabolic Regulation. (Dr. K.-P. Huang) studies the role of protein kinases and phosphorylation-dephosphorylation of proteins in the regulation of cellular functions. Also, the regulation and hormonal control of glycogen metabolism, and the activities of glycogen synthase and phosphorylase kinase. The receptor-mediated turnover of membrane phospholipids plays an important role in the regulation of many cellular functions. Two second messengers, inositol 1,4,5-trisphosphate and diacylglycerol, are generated from phosphatidylinositol 4,5-bisphosphate after cleavage by phospholipase C. Inositol 1,4,5-trisphosphate is believed to trigger the release of calcium from an intracellular nonmitochondrial pool, whereas diacylglycerol activates protein kinase C to modulate numerous cellular responses. This signal-transduction pathway has been implicated in the regulation of cell growth, differentiation, gene expression, hormone and neurotransmitter release, cell-surface receptor function, and cellular metabolism.

Protein kinase C is a  $\text{Ca}^{2+}$ /phospholipid-dependent enzyme which regulates cellular functions by phosphorylation of target protein substrates. This enzyme has also been identified as a receptor for tumor-promoting phorbol esters, which elicit pleiotropic physiological responses comparable to those by many hormones and growth factors. Three types of protein kinase C isozymes, designated type I, II, and III, have been purified to homogeneity from both rat and monkey brains. Polyclonal and monoclonal antibodies against rat brain protein kinase C were prepared and



applied to the immunochemical characterization of the enzyme from individual tissues. It was observed that protein kinase C from different animals, including humans, shared common immunoreactive determinants. By using antibodies specific for each isozyme, protein kinase C isozymes were shown to be present in several cell types and to be differentially distributed in individual brain regions. Based on the immunocytochemical localization of each protein kinase C isozyme in the various neurons where transcripts of distinct protein kinase C cDNAs have been identified by in situ hybridization, it was established that protein kinase C isozymes are products of discrete genes. This conclusion was further supported by immunochemical characterization of protein kinase C expressed in COS cells transfected with the various cDNAs.

The identification of the precursor/product relationship of the various cDNAs and protein kinase C isozymes establishes the molecular diversity of this enzyme family. The presence of multiple protein kinase C isozymes may be essential for this enzyme to participate in a variety of cellular functions in different tissues. Indeed, the various forms of protein kinase C isozyme do respond differently to its activators such as diacylglycerol and tumor-promoting phorbol esters, and they show different susceptibilities to proteolytic degradation. In addition, the various protein kinase C isozymes are expressed differently during development. The type I enzyme, which has been identified only in brain, was found to be expressed in correlation with brain synaptogenesis and myelination. In the monkey brain, this isozyme was found to be highly enriched in the temporal pole and entorhinal areas, both important regions for processing of visual information. These findings suggest that protein kinase C may be involved in higher mental functions such as learning and memory. In the lymphoid organs, such as thymus and spleen, the developmental expression of protein kinase C proceeds at a different rate compared with the brain, indicating unique developmental control in different tissues.

Protein kinase C plays a pivotal role in the regulation of gene expression. The expression of protein kinase C is in turn regulated by growth factors. Studies with fetal rat brain granule cells in culture revealed that the type I protein kinase C was slightly reduced while the type III enzyme progressively increased during culture in complete medium. A similar reciprocal mode of expression of the type II and type III protein kinase C isozymes was also observed during nerve growth factor-stimulated differentiation of PC-12 pheochromocytoma cells. The differential expression of protein kinase C isozymes during growth and differentiation may be an important determinant of unique cellular function at specific stages of development.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 HD 00022-14 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Renin-Angiotensin System and Aldosterone Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	G. Aguilera	Head	ERRB, NICHD
Others:	K. J. Catt	Head, SHR	ERRB, NICHD
	M. A. Millan	Sr. Staff Fellow	ERRB, NICHD
	S. Nakano	Visiting Fellow	ERRB, NICHD

## COOPERATING UNITS (if any)

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Endocrine Physiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to analyze physiological and pathological aspects of the renin angiotensin system, including the effects of AII in circulatory homeostasis, pituitary and gonadal function. AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomulosa zone to AII. Previous studies in the rat have demonstrated that the adrenal responsiveness to AII depends on the trophic effects of the peptide and the modulatory effect of other regulators such as dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF). Studies on the adrenal effects of SRIF were extended to the primate. SRIF like immunoactivity was identified in monkey and human adrenal cortex and the peptide was found to preferentially inhibit AII stimulated aldosterone production in isolated monkey adrenal cells, suggesting that SRIF has a role in the control of aldosterone secretion in primates. Studies on the mechanisms of action of aldosterone regulators were focused on the effects ANF in the adrenal and pituitary. ANF was a potent stimulatory of cGMP production but the cyclic nucleotide had no inhibitory effect on steroidogenesis. ANF inhibited aldosterone secretion without affecting AII induced increases in cytosolic calcium or phospholipid turnover. Arachidonic acid inhibited aldosterone secretion with characteristics similar to those of ANF suggesting that arachidonic acid metabolism may be involved in the effects of ANF. In pituitary cells ANF stimulated cGMP production without affecting basal or stimulated pituitary hormone production. In the gonads AII receptors are present in Leydig cells in the testes and granulosa and luteal cells in the ovary of rat and primates. In the ovary AII receptor activation by coupled to calcium mobilization with increases in cytosolic calcium. The presence of AII receptors in the testes and ovaries suggest a role for the peptide in the regulation of gonadal function.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00035-15 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Structure and Function of Biological Active Molecules

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. C. Chen	Head	ERRB, NICHD
Others:	J. L. Morell	Research Chemist	ERRB, NICHD
	J. H. Brown	Research Chemist	ERRB, NICHD
	T. C. Chang	Visiting Fellow	ERRB, NICHD
	F. A. Ghazanfari	Guest Researcher	ERRB, NICHD
	C. A. Owens	Guest Researcher	ERRB, NICHD

## COOPERATING UNITS (if any)

Human Genetics Branch, NICHD (M. Zasloff)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Structure and Protein Chemistry

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL:

1.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structural design, chemical synthesis, and modification of molecules important to reproductive and developmental biology are the focus of this study.

A. Broad spectrum anti-microbial substances (Magainins) isolated from skin of *Xenopus laevis* have been purified to homogeneity by reverse-phase high performance liquid chromatography. Amino acid sequence analyses by Edman degradation and carboxypeptidase Y digestion have revealed two sequences, magainin I and II which differ in two substitutions shown in parentheses for II: G-I-G-K-F-L-H-S-A-G(K)-K-F-G-K-A-F-V-G-E-I-M-K(N)-S. These sequences have been confirmed by both chemical synthesis and cDNA sequence analyses. Circular dichroism studies indicate that margainin peptides do not form  $\alpha$ -helices in aqueous buffers. However, magainin I and II display 24% and 26%  $\alpha$ -helical conformation in lipophilic solution (49% trifluoroethanol), respectively. These results indicate that the peptides are amphiphilic in structure which suggests that the lytic action of magainins may stem from a highly selective penetration into bilayers of microorganisms.

B. A peptide comprising the last 37 residues of human chorionic gonadotropin  $\beta$ -subunit has been synthesized and purified. Through the thiol function of Cys<sup>2</sup>, the peptide was coupled to a m-maleimidobenzoylated  $\epsilon$ -amino groups of Keyhole Limpet hemocyanin. This two-step conjugation approach yields a conjugate which contains 2:1 peptide to protein ratio (by weight) and permits the maximal exposure of antigenic sites.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00146-12 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Chorionic Gonadotropins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	H. C. Chen	Head	ERRB, NICHD
Others:	J. L. Morell	Research Chemist	ERRB, NICHD
	J. H. Brown	Research Chemist	ERRB, NICHD
	T. C. Chang	Visiting Fellow	ERRB, NICHD
	F. A. Ghazanfari	Guest Researcher	ERRB, NICHD
	C. A. Owens	Guest Researcher	ERRB, NICHD

## COOPERATING UNITS (if any)

Department of Chemistry, Georgetown University (D. C. H. Yang)  
Department of Health, State of New York, (N. J. Ellish)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Structure and Protein Chemistry

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

1.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the role of carbohydrate structures of human chorionic gonadotropin (hCG) in the subunit association kinetics, purification of pregnant mare serum gonadotropin, development of sub-nanomole sugar analysis of glycoproteins and of a highly specific and sensitive two sites immunoradiometric assay of hCG. A. The carbohydrate moieties in hCG are not essential for the association of subunits as studied by the enhancement of 1-anilino-naphthyl-8-sulfonate (ANS). They do influence the rate of association. The Scatchard analysis of hormone: ANS binding reveals that hCG and deglycosylated hCG (HF-hCG) give the following apparent dissociation constant ( $KD = \mu M$ ) and number of binding site ( $n$ ):  $KD=3.7$ ,  $n=1$  for hCG;  $KD= 2$  and  $8$ ,  $n=0.5$  and  $0.3$  for HF-hCG. Kinetic data indicates that the subunit association begins with a second-order rate ( $400 \text{ M-lmin}^{-1}$  for hCG and  $5700 \text{ M-lmin}^{-1}$  for HF-hCG) as the rate-limiting step followed by the slow first order conformational transition of the  $\alpha\beta$  complex. The influence of carbohydrate appears to reside at the first step. B. Three conventional column chromatography steps have been employed to obtain four potent fractions active in ovarian receptor binding. SDS-polyacryl amide gel electrophoresis has shown two bands in all four fractions which migrated at the positions of 25K and 48K daltons as compared to hCG at 24K and 35K. A sensitive and reliable method of monosaccharide analysis has been developed. The method is based on a reverse-phase high performance liquid chromatography to separate perbenzoylated methylglycosylic derivatives of sialic acids, N-acetyl-glucosamine, N-acetyl-galactosamine, galactose, mannose and fucose which are prepared from acid methanolysis of glycoproteins followed by benzoic anhydride treatment. C. A polyclonal antibody based on two-site immunoradiometric assay for hCG has been developed. The method is highly specific and sensitive for hCG assay in biological fluids, and is devoid of false positive derived from protease activities. Our system is capable of monitoring a wide variety of hCG molecules and potentially important for laboratory and clinical investigation.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00147-12 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Mechanism of Action of Peptide Hormones in Steroidogenic Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.L. Dufau	Head	ERRB, NICHD
Others:	C-H. Tsai-Morris	Staff Fellow	ERRB, NICHD
	C. A. Winters	Chemist	ERRB, NICHD
	A. Khanum	Visiting Fellow	ERRB, NICHD
	T. Minegishi	Visiting Fellow	ERRB, NICHD
	Maria Nishihara	Visiting Fellow	ERRB, NICHD
	Andrea Fabbri	Guest Researcher	ERRB, NICHD

## COOPERATING UNITS (if any)

Contract for preparation of gonadal cells and cell fractions to HD-6-2904

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.75

## PROFESSIONAL:

0.50

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Stimulation of the androgen pathway occurs mainly through cAMP mediated mechanism. The stimulatory event can be negatively influenced by the action of certain hormones. In recent studies we have demonstrated the presence functional angiotensin II (AII) receptors in the Leydig cell of high affinity  $K_a$  1.7 nM and low capacity 2,000 sites per cell. AII inhibits GTP and LH stimulated adenylate cyclase in Leydig cell membranes. This hormone also acutely inhibits LH stimulation of cyclic AMP pools and testosterone production in Leydig cells. These effects were prevented by incubation with pertussis toxin and reversed by 8-bromo cAMP additions indicating that AII action in the Leydig cell occurs through the guanyl nucleotide inhibitory unit of adenylate cyclase (Gi), findings that have further emphasized the importance of the cAMP pathway in the Leydig cell. In addition to the potentiation and direct stimulatory effect of forskolin, a dose-dependent inhibitory effect of forskolin (ID<sub>50</sub>, pM) on basal and hCG-stimulated cyclic AMP pools and testosterone production were demonstrated through inhibition of adenylate cyclase via direct or indirect activation of Gi. High affinity action of forskolin is of value for direct evaluation of functional Gi activity. Unlike the adult Leydig cell, the fetal and immature cell are refractory to desensitization and maintain up-regulated LH receptor and steroidogenic functions, and their inability to be desensitized by gonadotropin is attributed to the absence of an estrogen-mediated regulation of the androgen pathway. The fetal testis possess in addition to the predominant fetal cell population a small population of transitional cells with functional capacities of the adult cell. After appropriate treatment of fetal cultures (i.e. estrogen, and frequent or a high gonadotropin dose) emerge a functional adult-like cell type from the fetal Leydig cell population. The cultured fetal Leydig cell system provides a useful model to elucidate LH, GnRH action,  $\beta$ -endorphin regulation and the mechanism involved in the development of gonadotropin-induced estradiol-mediated desensitization of steroidogenesis.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00149-12 ERB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.L. Dufau Head ERRB, NICHD

Others: K.J. Catt Head, SHR ERRB, NICHD

## COOPERATING UNITS (if any)

Dept. Medicine, Charlottesville, VA, Dept. of Pediatrics, Contract for prep. of gonadal cells and cell fractions HD-6-2904.

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.25

## PROFESSIONAL:

0.25

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

In 1975 we developed a LH bioassay term RICT (rat interstitial cell testosterone) for measurement of circulating LH, 5-fold more sensitive than conventional radioimmunoassay. We have now developed a simplified and rapid method of comparable sensitivity for the measurement of LH/hCG in plasma, tissue extracts or incubation media. This bioassay uses microtiter plates and the product is, measured by an enzyme-linked immunoassay procedure using transfer solid phase. The procedure can be carried out in less than five hours (versus > 24 hr for RICT) with minimal reagent preparation. The principle and steps presented can be used also for the assay of pituitary and hypothalamic hormones or any protein hormone that can stimulate the release of a cell product which can be measured by the present approach (ie. steroid, cyclic nucleotide, gonadotropin).

We used RICT assay to assess biological LH activity secreted in response to endogenous and low dose exogenous GnRH pulses in normal men. The absence of non-specific plasma effects in the LH bioassay was demonstrated by the finding of undetectable levels of LH bioactivity despite low but measurable immunoactivity in 10 hypogonadotropic men. In normal men exogenous low dose (10 µg) i.v. GnRH administration resulted in preferential release of bioactive LH, with a consequent significant increase in the median plasma bio- to immunoactive LH ratio. This pattern mimicked that of endogenous LH pulsatility. The preferential increase in bioactive plasma LH in response to exogenous or endogenous GnRH might reflect the smaller initial distribution volume and slower metabolic clearance rate of bio versus immuno LH. We have demonstrated a sustained inhibitory actions on bio and immunoactive LH of a potent GnRH antagonist (N-acetyl-D-pCl-Phel,2-D-Trp3-D-Ala10GnRH10) in postmenopausal women, and shown that the GnRH antagonist binds avidly to serum proteins and has a prolonged plasma residence time. This may explain the observed extended duration of the antagonist action in vivo.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00150-12

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.L. Dufau Head ERRB, NICHD

Others: C. Winters Chemist ERRB, NICHD  
S. Kusuda Visiting Associate ERRB, NICHD  
T. Minegishi Visiting Fellow ERRB, NICHD  
D. Pineda Adjunct Scientist ERRB, NICHD

## COOPERATING UNITS (if any)

Contract for preparation of gonadal cells and cell fractions HD-6-2904

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL:

1.5

## OTHER:

0.50

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously purified the LH/hCG receptor of the rat ovary. The purified receptor was identified as a single protein ( $M_r=75,000$ ) on SDS-PAGE under reducing condition by silver staining. The homogeneity of the purified receptor was recently confirmed by microsequencing. Autoradiographic analysis of SDS-PAGE of labeled hCG (with label only in  $\alpha$ -subunit) crosslinked to pure receptor showed two radioactive bands of  $M_r=134,000$  and  $97,000$  which corresponded to the receptor hCG  $\alpha\beta$  complex and  $\alpha$ -subunit respectively. The above was confirmed using hCG cross-linked among subunits instead of native labeled hCG. Taking into account the contribution of hCG or its  $\alpha$ -subunit, the  $M_r$  of the receptor was calculated to be  $79,000$ . Cross-linking studies performed after binding to reconstituted hCG (radiolabeled in the individual subunits) to the purified LH/hCG receptor indicated that the hCG  $\alpha$ -subunit undergoes predominant interaction with the receptor molecule. For further characterization, the purified receptor was radioiodinated. Autoradiography of SDS-PAGE showed a single band of  $M_r=78,000$ . These values were consistent with those obtained for protein bands from unlabeled receptors and by cross-linking of hormone-receptor complexes. Treatment of labelled receptor with glycosydases demonstrated that the receptor molecule is predominantly N-linked glycosylated. Comparison of  $M_r$ 's derived from SDS gels with those from fast performance liquid chromatography suggested that the native LH holoreceptor is present in a dimeric form. Labelled hormone bound to non-denatured blotted monomeric and dimeric receptor forms. The Leydig cell receptor was also purified to homogeneity and shown to be of  $M_r 90,000$ , and appeared to be associated in dimers of identical subunits. It is likely that receptor dimerization and possible further aggregation are necessary for signal transduction, and receptor phosphorylation by one or more kinases may be involved in regulating gonadotropin action.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00151-12 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Gonadal and Placental Function

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. J. Catt Head ERRB, NICHD  
M. Knecht Sr. Staff Fellow ERRB, NICHD

Others: P. Feng Visiting Fellow ERRB, NICHD  
C. Das Guest Researcher ERRB, NICHD  
M. Zilberstein Research Associate ERRB, NICHD

## COOPERATING UNITS (if any)

Laboratory of Chemoprevention, Division of Cancer Etiology, NCI (M. Sporn).  
Department of Clinical Chemistry, University of Helsinki, Finland (I. Huhtaniemi)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.25

## PROFESSIONAL:

4.25

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In studies on the molecular basis of hormone action during granulosa cell differentiation, emphasis was placed on the functions and mechanisms of action of growth factors and plasminogen activator. TGF- $\beta$  exerted bifunctional actions on the maturation of granulosa cells, and altered the stimulation of cAMP formation, steroidogenesis, and LH receptor expression by FSH in a concentration-dependent manner. TGF- $\beta$  amplified gonadotropin responses in the presence of small amounts of FSH, but had less effect or even inhibited FSH action when FSH levels were elevated. The inhibitory effects of TGF- $\beta$  were observed only in the presence of insulin, suggesting that the total complement of hormones and growth factors within ovarian follicles determines the eventual development of granulosa cells. TGF- $\beta$  also modified EGF action during granulosa cell maturation through direct effects on EGF receptors. FSH increased EGF receptors during granulosa cell differentiation through elevations in cAMP levels. TGF- $\beta$  augmented the effects of FSH on EGF receptors, as well as increasing these binding sites in the absence of gonadotropin. The enhancement of EGF receptors by TGF- $\beta$  resulted in a parallel rise in the inhibitory effects of EGF on FSH-induced cAMP production and LH receptor formation. In studies designed to characterize specific proteins induced by FSH in granulosa cells, gonadotropin was shown to stimulate the production of a cell-surface tissue-type plasminogen activator, while both FSH-treated and control cells synthesize intracellular urokinase-plasminogen activators. Hormonal regulation of the production and activities of these enzymes may allow the expression of specific differentiated function of granulosa cells. In cultured human syncytiotrophoblasts from term placentae, the anti-progestin RU486 was shown to inhibit production of hCG, hPL, and progesterone. Also, arachidonic acid was found to be an exceptionally potent stimulus of hCG and hPL production, with effects in the nanomolar concentration range. It appears likely that placental hormone secretion, like pituitary hormone release, is influenced by lipoxigenase products of arachidonic acid.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00184-09 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Pituitary Hormone Secretion

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. J. Catt Head ERRB, NICHD

Others: M. L. Dufau Head, MES ERRB, NICHD  
G. Aguilera Head, SEP ERRB, NICHD  
R. O. Morgan Staff Fellow ERRB, NICHD  
J. P. Chang Guest Research ERRB, NICHD  
K. Tasaka Visiting Fellow ERRB, NICHD  
S. Stojilkovic Guest Researcher ERRB, NICHD

## COOPERATING UNITS (if any)

Department of Anatomy, University of Texas Medical Branch, Galveston, Texas (G. Childs); Contract for preparation of adrenal and pituitary cells NO1-HD-0-2806.

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

4.5

## PROFESSIONAL:

4.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The hypothalamic control of reproductive function is expressed through the receptor-mediated actions of GnRH on the pituitary gonadotroph. GnRH regulates gonadotroph function and LH secretion by binding to high affinity receptors in the plasma membrane. GnRH receptors appear to be confined to the pituitary and placenta in primates, but are present in gonads, brain, and other sites in the rat. The mechanism of receptor activation in gonadotrophs involves the integrated actions of several intracellular messenger systems. These include phosphoinositide breakdown and mobilization of intracellular calcium, as well as influx of extracellular calcium. In isolated gonadotrophs, GnRH stimulates the hydrolysis of phosphatidylinositol biphosphate to diacylglycerol and inositol trisphosphate (InsP<sub>3</sub>). The role of diacylglycerol and activation of protein kinase C in gonadotrophs has been suggested by studies on the translocation of protein kinase C and its regulation by activators (phorbol esters, synthetic diglycerides) and inhibitors (retinal). Also, the generation of IP<sub>3</sub> and promotion of calcium mobilization and entry provides a mechanism for the early elevation of [Ca<sup>2+</sup>]<sub>i</sub> during GnRH action. GnRH stimulates the production of several higher inositol phosphates (IP<sub>3</sub>, IP<sub>4</sub>, IP<sub>5</sub>) and causes marked elevation of Ins-4-P rather than Ins-1-P as the major product of polyphosphoinositide metabolism. Arachidonic acid (AA) and its lipoxygenated metabolites also mediate GnRH action, and are generated via activation of diacylglycerol lipase as well as phospholipase A<sub>2</sub>. The actions of AA on LH release are related to its effects on calcium mobilization and activation of an AA-dependent protein kinase in pituitary cytosol. The role of calcium entry in GnRH action is related to the time course of the LH response, which is at first independent of extracellular calcium but is subsequently dependent on calcium influx during the sustained phase of LH release in GnRH-stimulated gonadotrophs.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00187-08 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Hormonal Regulation of Cellular Metabolism

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	K.-P. Huang	Head	ERRB, NICHD
Others:	A. M. Goheer	Senior Staff Fellow	ERRB, NICHD
	F. Huang	Expert	ERRB, NICHD
	H. Nakabayashi	Visiting Fellow	ERRB, NICHD
	Y. Yoshida	Visiting Fellow	ERRB, NICHD

## COOPERATING UNITS (if any)

Laboratory of Developmental and Molecular Immunity NICHD, NIH  
(E. Hanna); Laboratory of Cell Biology, MH, NIH (W. S. Young)  
Section on Growth Factors, NICHD, NIH (G. Guroff)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Metabolic Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

5.0

## PROFESSIONAL:

5.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C, a  $\text{Ca}^{2+}$ /phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those by many hormones and growth factors. Three isozymic forms of protein kinase C have been purified to near homogeneity from rat and monkey brains. Polyclonal and monoclonal antibodies against these enzymes were prepared for the immunochemical characterization. These enzymes were found to be differentially distributed in various brain regions and each isozyme appears to be present in distinct neurons. Based on the immunocytochemical localization of each protein kinase C isozymes in the various neurons where transcript of distinct protein kinase C cDNA has been identified by in situ hybridization, we have established that protein kinase C isozymes are products of discrete genes. The molecular diversity of protein kinase C family may be essential for this enzyme to participate in a variety of signalling pathways in different tissues. Indeed, the various form of protein kinase C isozymes do respond differently to its activators such as diacylglycerol and tumor-promoting phorbol esters. The various forms of protein kinase C isozymes are expressed differently during development. The developmental control of protein kinase C gene expression, and the role of these enzymes in the regulation of various cellular functions, are currently under investigation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00190-05 ERRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adrenocortical Zonation: Regulation of Steroidogenesis & Cholesterol Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. A. Strott Head ERRB, NICHD

Others: M. Kubo Visiting Fellow ERRB, NICHD

C. D. Lyons Bio. Lab. Tech. ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of action of ACTH is only partially understood. Adrenal steroid production is rapidly activated and deactivated ( $\sim 2$  min); such a process is considered too rapid to involve regulation at the level of translation. Regulation would, however, be compatible with protein modification. It is generally accepted that in the adrenal cortex ACTH stimulates membrane-bound adenylate cyclase activity which leads to an increase in intracellular cAMP and the activation of cAMP-dependent protein kinase followed by steroid synthesis. The role of other protein kinases such as  $\text{Ca}^{2+}$ -regulated kinases, however, is not well understood as yet. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone by a cytochrome P450 enzyme system. There is as yet no evidence that the P450 for cholesterol sidechain cleavage is subject to modulation by a phosphorylation-dephosphorylation mechanism. ACTH is known to activate systems which increase the availability of cholesterol for steroid production; and although the latter systems are known to be modulated by phosphorylation-dephosphorylation mechanisms, none is known to be regulatory. In the ACB laboratory, the guinea pig is used as an animal model to explore the responsiveness of different zones of the adrenal cortex to ACTH. In this model, ACTH does not stimulate cholesterol side-chain cleavage activity and steroid production in the inner zone, in contrast to the outer zone, while adenylate cyclase activation and cAMP formation are similar for the two zones. The activity of HMG-Co A reductase (rate-limiting in cholesterol synthesis) is stimulated by ACTH in the inner zone as it is in the outer zone. Examination of protein kinase activity and protein phosphorylation reveals that cAMP-dependent,  $\text{Ca}^{2+}$ /phospholipid-dependent, and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase activities are significantly higher in the outer zone than in the inner zone, and protein phosphorylation induced by the three kinase systems in the two adrenocortical zones reveals notable differences in phosphoprotein patterns.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00191-03

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Neuroendocrine Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Aguilera	Head	ERRB, NICHD
Others:	K.J. Catt	Head, SHR	ERRB, NICHD
	P. Carvallo	Visiting Fellow	ERRB, NICHD
	M.A. Millan	Sr. Staff Fellow	ERRB, NICHD

## COOPERATING UNITS (if any)

NIA, NIH	(J.P. Harwood)
Dept. of Psychiatry, UCSD	(R.L. Hauger)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Endocrine Physiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL:

1.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigation has focused on the regulation and actions of corticotropin releasing factor receptors and the interactions of CRF with other ACTH regulators.

A. CRF receptor regulation. We have previously shown that pituitary receptor downregulation and desensitization that accompany the increase in plasma ACTH often adrenalectomy is partially due to increased hypothalamic CRF secretion. Studies in rats with hypothalamic lesions demonstrated that the effect of adrenalectomy is completely dependent on hypothalamic factors. Glucocorticoid deficiency per se is not involved since physiological amounts of corticosterone decrease CRF receptors. Desensitization of plasma ACTH responses during prolonged stress is accompanied by CRF receptor downregulation and desensitization of cAMP responses to CRF. However, pituitary responsiveness in vivo is maintained or increased probably due to spared/CRF receptors and other regulators, mainly VP. Brain CRF receptors were unchanged during glucocorticoid administration and stress.

B. Mechanisms of action and interaction between ACTH regulators. Previous studies have shown that the effect of CRF is cAMP dependent while other stimuli increase ACTH secretion and potentiate the stimulation by CRF through calcium/phospholipid dependent mechanisms, with activation of protein kinase C. Further studies demonstrated that potentiation of CRF action by VP involves enhancement of CRF stimulated cAMP levels due to inhibition of phosphodiesterase, and protein kinase C dependent phosphorylation of a component of adenylate cyclase. Regulation of corticotroph function also involves dual effects of arachidonic acid metabolites, with lipooxygenase and cyclooxygenase products being stimulatory and inhibitory, respectively. Kinetic studies in cultured pituitary cells showed two phases of ACTH secretion by CRF and cAMP independent stimuli; an early phase with a rapid increase in ACTH release rate which is independent of extracellular calcium, and a late phase of constant secretion rate with partial calcium dependence for CRF, and complete calcium dependence for non-cAMP dependent stimuli.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00192-02 ERRB

PERIOD COVERED October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification, Immunology, and Functional Activity of Adrenocortical Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: C. A. Strott Head ERRB, NICHD

Others: Y. C. Lee Senior Staff Fellow ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The adrenal cortex of the guinea pig contains specific steroid-binding proteins which have been only partially purified and characterized; their function is as yet undetermined. For instance, there are proteins which specifically bind cholesterol, cholesteryl sulfate, pregnenolone, and pregnenolone sulfate. These proteins are of great interest because the rate-limiting reaction in steroidogenesis is the conversion of cholesterol to pregnenolone or cholesteryl sulfate to pregnenolone sulfate. The pregnenolone-binding protein has been determined to behave as a 58 kD protein by gel permeation chromatography and as a 34 kD species by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The 34 kD protein was electroeluted from acrylamide gels and injected into rabbits; an antibody was produced that specifically bound to and removed from solution pregnenolone-binding activity. When antibody-bound protein was eluted from the protein A-IgG column and re-examined by SDS-PAGE, a 34 kD protein was again generated. As a control, a 29 kD protein was simultaneously electroeluted and antibodies were raised against it. Unlike the situation with antibody to the 34 kD protein, antibody to the 29 kD protein did not interact with pregnenolone-binding activity. These results confirm that the pregnenolone-binding protein is a 34 kD protein as previously reported. Unfortunately, antibody titers to date are low and this has impeded progress, but efforts are being made to remedy this situation. In addition to the non-catalytic steroid-binding proteins, efforts are underway to isolate and produce antibodies to the cytochrome P450 for cholesterol side-chain cleavage. The latter antibody will be used to quantitate P450 in the outer and inner adrenocortical zones of the guinea pig. In this animal model cholesterol side-chain cleavage activity is stimulated in the outer zone but not the inner zone. The antibody to P450scc will also be used to isolate the mRNA for P450scc.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00193-02 ERRB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Angiotensin II Receptors and Activation Mechanisms

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	K. J. Catt	Head	ERRB, NICHD
Others:	G. Aguilera	Research Biologist	ERRB, NICHD
	T. Balla	Visiting Fellow	ERRB, NICHD
	G. Guillemette	Guest Researcher	ERRB, NICHD
	A. Baukal	Biomedical Engineer	ERRB, NICHD
	M. Carson	Guest Researcher	ERRB, NICHD
	W. Hausdorff	Guest Researcher	ERRB, NICHD
	C. Harper	Guest Researcher	ERRB, NICHD

## COOPERATING UNITS (if any)

Dept. of Physiology, Semmelweis University Medical School, Budapest (A. Spat)  
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

6.0

## PROFESSIONAL:

4.0

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The properties of angiotensin II (AII) receptors and their intracellular signaling pathways were studied in the adrenal zona glomerulosa and other target tissues. The mechanisms leading to stimulation of steroidogenesis were analyzed in isolated glomerulosa cells from the rat and bovine adrenal cortex. Purification of photolabeled AII receptors of the bovine adrenal gland was pursued by detergent solubilization and fractionation by ion exchange, lectin-affinity, and immunoaffinity chromatography. Elevation of cytoplasmic calcium by AII depends upon mobilization of intracellular calcium stores by the products of ligand-stimulated phosphoinositide turnover, and also on calcium entry through voltage-sensitive channels. Microsomal receptors for inositol-1,4,5-trisphosphate (IP3), previously identified in adrenal microsomes, were also demonstrated in the anterior pituitary gland. The Ins-1,4,5-P3 formed from PIP2 breakdown during AII action was rapidly eliminated via two metabolic routes. In addition to breakdown via Ins-1,4-P2 and Ins-4-P via the previously identified 4-monophosphate pathway, the calcium-mobilizing 1,4,5-trisphosphate isomer is rapidly converted to Ins-1,3,4,5-P4, which is then degraded to the inactive 1,3,4-trisphosphate isomer. The latter is metabolized by degradation to Ins-3,4-P2 and Ins-1,3-P2, and also undergoes a further cycle of phosphorylation to form a novel tetrakisphosphate isomer, recently identified as Ins-1,3,4,6-P4. These studies have further indicated the importance of the 4-monophosphate pathway in inositol polyphosphate catabolism, and have revealed new phosphorylation pathways and inositol metabolites with potential roles in intracellular signalling and AII action in the glomerulosa cell and other target tissues.

## HUMAN GENETICS BRANCH

- Z01 HD 00131-13 Human Biochemical Genetics  
William A. Gahl, M.D., Ph.D.
- Z01 HD 00133-10 Study of Glycogen Storage Disease  
James B. Sidbury, Jr., M.D.
- Z01 HD 00403-06 Magnesium Metabolism in Mothers and Neonates  
Joan L. Caddell, M.D.
- Z01 HD 00404-05 Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases  
Jean DeB. Butler, Ph.D.
- Z01 HD 00405-09 Structure of the Methionine Initiator tRNA Genes in the Human Genome  
Michael A. Zasloff, M.D., Ph.D.
- Z01 HD 00408-04 Pathophysiology and Treatment of Human Genetic Diseases  
Michael A. Zasloff, M.D., Ph.D.
- Z01 HD 00410-02 Metabolism in Children with Glycogen Storage Disease, Type I  
James B. Sidbury, M.D.
- Z01 HD 00411-02 Evaluation of Nalmefene, an Endorphin Antagonist, in the Control of Appetite  
James B. Sidbury, M.D.
- Z01 HD 00909-08 Fetal Alcohol Syndrome  
Anil B. Mukherjee, M.D., Ph.D.
- Z01 HD 00910-08 Molecular Biology, Biochemistry and Physiology of Endogenous Antiinflammatory Proteins  
Anil B. Mukherjee, M.D., Ph.D.
- Z01 HD 00912-08 Gene Regulation and Cellular Differentiation  
Janice Y. Chou, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Human Genetics Branch

The Human Genetics Branch conducts research which attempts to elucidate the pathophysiology of human genetic and developmental disorders through an understanding of basic biological mechanisms. Clinical activities includes studies of the natural history, treatment, and methods of diagnosis of several heritable disorders of man.

Section on Molecular Biology

During the past year work in the section directed by M. Zasloff has concentrated on several problems: the mechanism of processing of eukaryotic RNAs; the basic mechanism involved in the transport of RNA from nucleus to cytoplasm; the expression of ALU sequences in eukaryotes. Clinical research in the section has focused on processes and disorders related to bone formation in man; the pathophysiology of osteogenesis imperfecta; the expression of collagen genes in man; the structure of the bone-specific alkaline phosphatase gene in man.

A totally new area of research opened this past year. A novel family of anti-microbial peptides were isolated from the skin of *Xenopus laevis*, uncovering a previously unrecognized vertebrate host defense system.

Over the past several years, this group has studied the pathway of expression leading to biosynthesis of the tRNA<sup>met</sup> molecule in human cells. The studies have provided insights into the organization of the tRNA gene family in man and the pathways utilized in delivery of a mature tRNA into the cytoplasm of eukaryotic cells. The laboratory had previously shown that a naturally occurring tRNA<sup>met</sup> variant exhibited a defected phenotype in several in vitro systems. The group demonstrated that the natural variant gene contained a point mutation which resulted in the appearance of a primary gene transcript that was inefficiently processed. In addition, through the use of microinjection and micro-dissection methodology in the *X. laevis* oocyte system, the group demonstrated that the variant tRNA was defective in its transport from the nucleus to the cytoplasm. This discovery led to the first description of the mechanism by which an RNA is transported from the nucleus of a eukaryotic cell. It was demonstrated, in fact, that tRNA species were transported by a saturable, carrier-mediated mechanism. We proposed that a ribosome-like element at the nuclear envelope was the actual motor utilized in this process. To fully explore the domain of the tRNA<sup>met</sup> molecule recognized by the transport system, 30 point mutations were generated in the human gene by in vitro mutagenesis utilizing hydroxylamine. Analysis of the transport phenotypes obtained demonstrated the unexpected finding that the tRNA molecule is exquisitely sensitive to mutation in its transport properties. The particularly sensitive areas included the T and D loops of the tRNA, the most highly conserved portions of the species. Another feature of this study was the demonstration that every mutation which yielded a tRNA species defective in transport, also yielded a pre-tRNA species which was inefficiently processed in vivo. This correlation led to the postulate that the processing enzymes might in some manner be playing a role in tRNA transport. As a result, the processing nucleases were purified from eukaryotic cells, representing the first characterization of these classes of enzyme in eukaryotes. We demonstrated that two enzymes, both endonucleases, process the primary transcript of the human

tRNA<sup>met</sup> gene to a mature species. The first reaction involves cleavage of the 5' leader; the second, cleavage of the 3' trailer. Two enzymes have been purified from *X. laevis* oocytes and KB cells. The enzyme which processes the 3' trailer is a simple polypeptide of about 97,000. Its most striking feature is that it will only cut the 5' processed primary transcript, establishing a cutting order to the pathway. The first cutting activity falls into the lap of the 5' nuclease. This enzyme has been purified to homogeneity and appears to be one of the most complex enzymes yet described in animal cells. It is composed of at least 14 different polypeptides ranging in MW from 20,000 to 32,000. It has the shape of a cylinder, composed of a stack of 4 rings. The entire structure appears to be necessary for tRNA processing. The precise relationship between its structure and enzymatic activity is under study. It is curious that this particle has been described in the literature over the past 15 years, having been isolated from organisms ranging from *Drosophila* to man. Until our report, its function remained a mystery. The relationship between this particle and tRNA transport remains to be ascertained.

The pathway of expression of a naturally occurring eukaryotic nonsense opal suppressor tRNA was elucidated this past year. This tRNA, which donates a phosphoseryl residue, is present in single copy in eukaryotes from yeast to man. We have shown that the gene is transcribed in vitro and in vivo to yield a primary transcript initiating at the nucleotide corresponding to the mature 5' terminus of the cytoplasmic tRNA. This 5' terminus retains its triphosphate residue while the 3' trailer is cleaved endonucleolytically. By using the highly purified processing endonucleases described above, we demonstrated that the primary transcript of this gene is 3' processed by the activity which acts on other cytoplasmic species and does not require prior 5' maturation. The absence of 5' processing distinguishes this tRNA biosynthetic pathway from all others previously described. The significance of this deviation is unclear, but may reflect the unusual role this tRNA may play in cellular function. It is striking that the maturation pathway of this species superficially resembles that of the Alu sequence.

Work continued on analysis of the mechanism of mRNA transport. These studies utilized the *X. laevis* oocyte system which was previously exploited to define the mechanism of tRNA transport. The mRNA species studied was transcribed from the Herpes thymidine kinase gene. We established that kinetics of TK mRNA transport could be studied in this in vivo system after introduction of the gene into the oocyte nucleus. We made the surprising discovery that the transport behavior of mRNA in the nucleus of this cell could be dramatically altered by the quantity and nature of DNA sequences introduced, in trans, to the RNA transcribed in the cell. These experiments demonstrated that sequences which mapped to the promoter of the TK gene could "activate" transport when introduced into the nucleus of the oocyte. Precise mapping of the critical regions within the promoter identified the TATA homology as particularly critical for this effect. The studies suggested a novel role for the promoter of a eukaryotic gene: the interaction of the sequence with intranuclear components necessary for disposition of mRNA transcribed from that gene from nucleus to cytoplasm. Just as these sequences may facilitate interaction with RNA polymerase II they may facilitate interaction of the gene with transport-determining components in the nucleus. These studies identify a totally novel level of potential control and regulation over gene expression.

We have continued our study of the Alu sequence family over the past year.



These sequences comprise around 3-6% of the vertebrate genomes, present in about 300,000 copies. We have demonstrated that one such sequence, the murine B1 sequence, is processed and transported from nucleus to cytoplasm. The particular sequence studies lies antisense to a murine gene encoding alpha-fetoprotein (AFP). The processing reaction involving the Alu primary transcript appears to be endonucleolytic, releasing the 3' trailer. The "core" Alu RNA generated is transported upon processing into the cytoplasm. We have shown that this species is present in highest abundance in murine fetal liver, the tissue in which AFP is most actively expressed. This result was unexpected since current thinking would assign an inhibitory role to this natural antisense RNA. The Alu sequence RNA, in addition, was shown to be associated with a specific polypeptide of about 63,000 in MW, a polypeptide identified through the use of an autoimmune antiserum from a patient with lupus. This polypeptide appeared to be associated with the primary transcript of the Alu sequence in its nuclear phase, and remained associated after processing and transport to the cytoplasm. The affinity purified antibody further identified a protein of similar size in KB cells. Associated with this polypeptide in the human cells was a small RNA of about 80 nt. We suspect that this RNA will represent the human analogue of the B1 Alu family. It suggests that, despite nucleic acid sequence divergence, the Alu sequence between vertebrates may be functionally conserved. Its role in gene expression several systems is under study.

These studies on the Alu sequence were extended to a related reiterated small sequence, the "identifier" sequence, previously suggested to represent brain-specific RNA species. The identifier sequences are about 80 nt RNA species, with considerable homology to tRNA species. They are found in many eukaryotic transcription units and are transcribed by polymerase III. We have found that they undergo a distinct processing and transport pathway very similar to the pathway deduced for the Alu sequence family. At least 6 or so identifier sequences were studied by transcription in *X. laevis* oocytes of cloned sequences subcloned from different mammalian genes. Each was transcribed to yield a long primary transcript and processed by a single 3' endonucleolytic cleavage to yield an 80 nt RNA. 5' to the site of cleavage in each case a stem-loop structure could be identified. Processing was found to be obligatory for entry of these RNA species into the cytoplasm. Present studies are designed to determine the extent to which the expression of these RNA species varies between tissues and developmental states. As with the Alu sequences a function for these sequences remains a major question to be answered.

The pathophysiology of hypophosphatasia was investigated at a molecular genetic level. Over the past year the cDNA species for alkaline phosphatase from bone were cloned from bovine and human osteoblast cDNA libraries. Almost a complete sequence of the bovine species has been obtained and partial data is emerging with respect to the human. The basic goal of these studies is to identify the bone-specific mRNA for alkaline phosphatase from a normal bone tissue to begin dissection of the role the enzyme plays in bone formation and development.

Studies on the pathophysiology and treatment of osteogenesis imperfecta continue. After considerable effort, the transcription units for alpha-2 type I collagen was determined in both skin and osteoblasts of avian and human origin. In both cases the mRNAs do not undergo alternative splicing or variations in promotor usage in bone and skin fibroblastic cells. This result suggests that other mechanisms must operate to explain the considerable greater abundance of collagen-specific mRNA in the osteoblast, including tissue-specific enhancers, splicing alternatives 3',

differential stability, etc.

Techniques for identification of mutation in the collagen polypeptide genes were continued. These included mRNA/DNA mismatch detection, polypeptide synthesis in culture, thermal stability of secreted procollagen, and restriction length polymorphism. It is expected that, as mutations are identified, they will be used to extend our understanding of the role of collagen structure in bone development.

Clinical studies in O.I. have dealt with the use of lower limb bracing in children with O.I. and the hormonal basis of growth failure in certain affected children. The bracing study has asked whether support of the lower limbs accelerates walking in affected children. This is important since weight bearing leads to enhanced mineralization and more dynamic modeling of the fragile lower limb skeleton. Initial results are exciting and will almost certainly lead to the application of these bracing techniques in this disorder. The second study attempts to determine if a defect in the known growth-promoting hormones is correlated with growth failure in O.I. It appears that growth failure is seen variably in affected individuals, unrelated to severity of bone fragility. The current studies involve evaluation of growth hormone secretion and IGF I and IGF II levels, both steady state and provoked. Initial studies suggest that children with severe O.I. may have extremely low levels of circulating IGF I, suggesting a role of this hormone in the process. The apparent decrease in GH secretion in some affected patients has prompted the use of clonidine as a stimulant of endogenous secretion.

Over the past year a very novel and somewhat unexpected area opened up and will most certainly occupy increasingly more effort over the coming years. It was noted that the frogs used as the source of oocytes in many of the experiments described above rarely became infected at the sites of surgical incisions placed after removal of the ovaries. It appeared as if these animals sterilized the wound sites without utilizing components of the classical immune system. As a result, a determined effort was made to identify anti-microbial substances in the skin of *Xenopus*. Indeed, such substances were readily identified and characterized. Two major peptides, each 23 residues in length, were initially recovered. We called them Magainins, reflecting their role as potential "shielding" substances in this animal's anti-microbial defense. The peptides display a potential amphiphilic character when configured as an alpha helix. They differ from each by 2 residues. The peptides act on a very wide spectrum of organism, including bacteria, fungi, and protozoa. Although the mechanism of action is still under study, it was evident very early that these substances profoundly affect membrane functions of susceptible organisms in that they rapidly induce osmotic lysis of protozoa by inhibiting contractile vacuole function. Unlike bacterial lysins, these peptides show a striking selectivity. they exhibit essentially no hemolytic activity and do not degranulate rat or human mast cells. The basis remains unclear. The cDNA has been cloned from *Xenopus* skin and it appears that both magainin 1 and 2 derive from a common precursor protein. They are both cleaved proteolytically at classical cleavage sites. By in situ hybridization we have shown that these peptides are produced in the so called "granular glands" of the skin, structures which also contain numerous peptides found in mammalian neurosecretory cells. Current studies are directed to analysis of mechanism of the magainins, identification of mammalian analogues, dissection of the physiology, developmental and molecular biology of these peptides in *Xenopus*, and physical properties of these peptides as they relate to structure and function. Studies are underway to



define their therapeutic potential in the treatment of human disease.

### Section on Developmental Genetics

This Section conducts both basic and clinical research in order to understand (i) the biochemical and molecular mechanism(s) of action and genetic regulation of steroid-induced phospholipase A<sub>2</sub> (PLA<sub>2</sub>) inhibitory, antiinflammatory proteins, and (ii) the pathogenesis of intrauterine growth retardation and genetic predisposing factor(s) in Fetal Alcohol Syndrome (FAS).

For the past several years this Section has been interested in a fundamental question in biology, namely, how the mammalian organism prevents inflammatory reactions in the vast mucosal epithelium which lines the passageways of all organs which communicate with the external environment. This is a fundamental problem, because this wet mucosa encounters myriads of foreign antigens and yet, under normal circumstances, no allergic or inflammatory response is elicited against these antigens. It has been suggested that there may be endogenous antiinflammatory agents under steroid hormonal control which may be responsible for this homeostasis. Although a specific endogenous antiinflammatory agent has not yet been identified for protecting the mucosal epithelium, there are several proteins which might be possible candidates. These proteins are phospholipase A<sub>2</sub> inhibitors and probably down-regulate the arachidonate cascade and thus, lower the tissue eicosanoids which are thought to be the mediators of inflammation.

Some of these inhibitors are structurally related and are collectively known as lipocortins. This Section has been working on a low molecular weight secretory protein, first discovered in the rabbit, variously known as blastokinin or uteroglobin (UG). During the years Dr. Mukherjee and his associates have shown this protein to be a very potent immunomodulator and an antiinflammatory agent. More recently, they have demonstrated that UG is an extremely potent PLA<sub>2</sub> inhibitor, although genetically distinct from lipocortins. Because of their similarity in function (i.e., PLA<sub>2</sub> inhibition), the structures of these proteins have been compared by using several computer programs, e.g., PRTALN, FASTP, RDF and HYDRO. This group has discovered that there is a considerable peptide sequence homology between lipocortin I and II and UG and a striking similarity in the hydropathy profiles of UG, the corresponding regions of lipocortins and the PLA<sub>2</sub>. Based upon these results, these investigators have custom synthesized peptides of these homologous regions and tested for their PLA<sub>2</sub> inhibitory activity. It was found that these synthetic peptides are 1000 times more potent PLA<sub>2</sub> inhibitors than the protein itself. Peptides derived from the non-homologous regions are also tested as controls and found to be inactive in PLA<sub>2</sub> inhibitory activity. Substitution of various amino acids in these peptides also cause loss of its inhibitory activity. These results suggest that these novel peptides may indeed be the active regions of these proteins responsible for their observed PLA<sub>2</sub> inhibitory property. These peptides have been tested for their effect on carrageenin and phorbol myristate acetate-induced inflammation in rat paws and rabbit skin respectively and found to be extremely potent antiinflammatory agents *in vivo*. Since both carrageenin and phorbol induce inflammation via PLA<sub>2</sub> activation, the antiinflammatory effects of these peptides seem to be the inhibition of PLA<sub>2</sub> and consequent reduction of tissue eicosanoid levels.

This Section has demonstrated that UG is present in all wet mucosal epithelium of the rabbit and also present in the venous circulation proximal to the mucosa



where it is known to be synthesized (e.g., uterus and tracheobronchial tree). UG is regulated by different hormones in different organs. For example, UG in the uterus is regulated by different hormones in different organs. For example, UG in the uterus is regulated by progesterone and in the respiratory epithelium it is controlled by corticosteroids. This multihormonal control of UG gene makes it more interesting from the standpoint of genetic regulation studies. To this end, Dr. Mukherjee and his colleagues have been successful in establishing cell lines from all organs of the rabbit which synthesize UG. These cell lines are valuable from the standpoint of studying the regulation of UG gene and steroid hormone action in vitro. Additionally, these investigators have obtained the cDNA probes for the UG and PLA<sub>2</sub> genes, synthesized a lipocortin-specific oligonucleotide probe and are at present investigating the expression of these genes in the established epithelial cell lines in order to delineate the temporal relationship of expression of these genes and the influence of different steroid hormones on them. Furthermore, the possibility that these cell lines may provide an in vitro model system for studying the biological potency of various progestogenic agents is now being investigated. In addition, these cell lines provide an in vitro test system for various progestogenic agents: synthetic or natural.

Because of the fast growth rate, endometrial origin and having a biochemical marker (i.e., UG) these cell lines may be valuable for developing a model for endometriosis. The development of this animal model is now being attempted. If successful, this model for the first time will enable investigators to test various avenues of therapeutic intervention in this disease.

Because of its potential application as an antiinflammatory/immunomodulatory agent and also because of its importance as a model system to study steroid hormone action at the cellular and molecular level this Section has undertaken investigations to express the UG gene in bacterial hosts. During the past year they have been successful in expressing this gene in E. coli although further improvements are now being made to enhance the expression so that large scale purification of recombinant UG can be undertaken.

The search for genetic predisposing factors in Fetal Alcohol Syndrome (FAS) has been continued. Two patients were admitted at the Clinical Center with the diagnosis of FAS under the clinical protocol 83-CH-228. One additional patient with the same diagnosis was also seen at the outpatient clinic of Howard University Medical Center. Skin biopsies from two of these patients were obtained and fibroblast cultures were established. These cells were used for transketolase enzyme kinetics. Preliminary results indicate an abnormal  $K_m$  for TPP for one of these patients while the second patient's  $K_m$  was within normal limits. Three age and sex matched control patient's fibroblasts were also studied and all had normal  $K_m$  for TPP for this enzyme. A three year review of this protocol has been completed and the ICRS has approved it for three additional years because of the difficulty in recruiting patients with FAS. The purification of human transketolase enzyme and the cloning of the transketolase gene are now being contemplated.

### Section on Disorders of Carbohydrate Metabolism

The study of the response of patients deficient in glucose-6-phosphatase to the administration of different corn starches continues. Using cooked and uncooked starches which have been tested in vivo, we have measured amylase activity in vitro with commercial amylase and the sera of patients tested prior in vivo. We did not get the expected correlation of in vivo with in vitro response.

The liver glucose production rates have been determined in 4 patients with type I glycogen storage disease and one with type III. We have verified the production of glucose by individuals with deficient glucose 6-phosphatase activity. We can distinguish the rate of production in those with total absence from those with partial deficiency.

An assessment of the potential efficacy of nalmefene, a third generation compound, in the control of appetite in the Prader-Willi syndrome and found it totally wanting. The protocol has been discontinued.

Work continues in the study of the mechanism of SIDS, using the magnesium-deficient rat as the animal model. The lung pathology, light and EM visualization shows striking similarity with that found in SIDS death infants. It was shown that the audiogenic seizure-shock episode in the Mg-deficient rat is associated with a massive catecholamine release (as well as other putative neurotransmitters). These episodes can be aborted or prevented by the prior administration of magnesium and glucocorticoids or with ibuprofen alone. A thromboxane-A<sub>2</sub> receptor blocker significantly modifies the seizure.

### Section on Cellular Differentiation

The studies in the section directed by Janice Chou have concerned regulation of gene expression during normal and abnormal differentiation processes. Studies on expression of the  $\alpha$ -fetoprotein (AFP) gene in temperature-sensitive (ts) fetal liver cells were continued. Chou and her coworkers have demonstrated that transformed fetal hepatocytes produce a 65K variant AFP which is encoded by a mRNA of 1.7 kb. Normal fetal liver produces two AFP species of 69K and 73K (fetal AFP) which are encoded by a mRNA of 2.2 kb. They have characterized the 1.7-kb RNA by a combination of northern-blot hybridization, nuclease S1 analysis, primer extension, and hybrid selected translation experiments. They found that the 1.7-kb AFP mRNA lacks the first seven 5' coding exons present in the 2.2-kb RNA. However, the two mRNAs have identical coding sequence from the eighth exon (corresponding to nucleotide 873 of the 2.2-kb fetal AFP mRNA) to the 3' end. The 1.7-kb variant AFP mRNA contains additional sequence 5' to the eighth exon and the 5' terminus of this mRNA are heterogeneous.

The biochemistry of liver maturation was studied using the RLA209-15 fetal rat hepatocyte line that is ts for maintenance of the differentiated fetal liver phenotype. Chou's group found that administration of glucocorticoid hormones to these cells at 40°C (nonpermissive temperature, differentiated phenotype) induced a series of events associated with normal hepatocyte maturation; synthesis of fetal AFP was inhibited whereas synthesis of albumin, transferrin, TAT, and  $\alpha$ 1-acid glycoprotein (AGP) was induced. Normal adult liver produced three AFP mRNAs of 2.2 kb, 1.7 kb, and 1.6 kb. The 1.7-kb adult liver RNA was indistinguishable from the 1.7-kb AFP mRNA found in RLA209-15 fetal hepatocytes.

In collaboration with Dr. G. Yeoh of Western Australia, Chou studied regulation of tyrosine aminotransferase (TAT) gene expression in an adult rat hepatocyte line, established in Chou's laboratory, which is ts for maintenance of differentiated liver phenotype. Glucocorticoid hormone was found to be necessary for expression of the TAT gene. In the absence of this steroid, enzyme synthesis, activity, and mRNA accumulation was virtually abolished. In addition, expression of TAT gene was ts and expressed mainly at the nonpermissive temperature when these cells have a nontransformed phenotype. cAMP alone was not sufficient to induce expression of the TAT gene, but it enhanced the induction caused by glucocorticoid. Immunocytochemical studies revealed that the enhanced expression of the TAT gene at the nonpermissive temperature and in the presence of glucocorticoid or glucocorticoid plus cAMP resulted from an increase in both the number of cells producing this enzyme and the quantity of TAT synthesized per cell.

Chou and her coworkers have isolated and characterized two cDNA clones of 1909 bp (PSG16) and 2128 bp (PSG93) encoding human placental pregnancy-specific  $\beta$ 1-glycoprotein (PS $\beta$ G). The sequenced coding and 3' ends of the 3' untranslated regions of these two cDNAs are identical. However, PSG93 contains an additional 86 bp at the end of the common 3' coding region. This insertion could result in the generation of PS $\beta$ G species of 418 amino acid residues instead of the 416 amino acid residues predicted by the sequence of clone PSG16. Two placental PS $\beta$ G mRNAs of 2.2 kb and 1.7 kb have been identified. Primer extension and S1 nuclease analysis demonstrated that the PS $\beta$ G mRNA has heterogeneous 5' ends.

The PS $\beta$ G cDNA probe isolated by Chou's group was used to examine regulation of PS $\beta$ G synthesis in human placental fibroblasts which produce this protein ectopically. They found that the fully processed PS $\beta$ Gs synthesized by fibroblasts and normal placenta were 63K and 72K, respectively, suggesting structural divergence. The structural difference of these two PS $\beta$ G species was confirmed by in vitro translation of PS $\beta$ G directed by poly(A)RNA isolated from fibroblasts or human placenta. Placental fibroblast RNA directed the synthesis of a single 46K polypeptide, whereas human placental RNA directed the synthesis of three polypeptides of 50K, 48K (major), and 36K. Although placental fibroblasts produced a variant PS $\beta$ G, authentic PS $\beta$ G could be produced by these fibroblasts in the presence of sodium butyrate. Butyrate stimulated de novo synthesis of PS $\beta$ G in placental fibroblasts and the butyrate-mediated induction is regulated at the pre-translational level.

#### Section on Human Biochemical Genetics

The Section on Human Biochemical Genetics investigates the clinical and basic research aspects of inborn errors of metabolism in man. Specific areas of pursuit are disorders of lysosomal membrane transport.

The Section has described the biochemical errors in both cystinosis and Salla disease, the only two lysosomal storage disorders due to impaired transport of small molecules out of lysosomes. Cystinosis, due to defective cystine egress, was shown for the first time to result in the storage of cystine within sorted, cultured cystinotic myotubes. Lysosomal cystine transport was also shown to be impaired in fibroblasts from patients with I-cell disease (mucopolidosis II), as well as unusual variants of cystinosis. Studies of free sialic acid, whose transport out of lysosomes is defective in the Finnish disorder, Salla disease, were also carried out. Lysosomal transport of this charged sugar was also found to be impaired in



infantile free sialic acid storage disease, a more severe variant of Salla disease.

Members of the Section described a lysosomal transport system for tyrosine and other neutral amino acids in rat FRTL-5 thyroid cells. The system represents the first lysosomal membrane carrier shown to be responsive, being stimulated by thyroid-stimulating hormone. This is in contrast to the hormonally unresponsive cystine carrier in FRTL-5 cells. The cystine carrier in these cells requires protein synthesis, since its activity is inhibited by cycloheximide and actinomycin D, but does not require N-linked glycosylation for activity, since tunicamycin, castanospermine, and deoxymannojirimycin do not inhibit it.

This year the Section described a new high performance ion exchange chromatographic technique for the separation of proteoglycans. In addition, the sulfation and synthesis of proteoglycans was analyzed in several fibroblast strains from normal individuals and patients with Lowe (oculocerebrorenal) syndrome. This disorder is characterized by X-linked inheritance, congenital cataracts, mental retardation, and renal Fanconi syndrome. Proteoglycan synthesis was found to be normal in Lowe syndrome fibroblasts, but the enzyme nucleotide pyrophosphatase was 3 to 8-fold elevated. This finding verifies that an increased enzyme level is a clue worthy of further pursuit in searching for the cause of this disease.

In clinical studies, the Section cares for 40 to 50 patients with cystinosis, by far the largest group of such patients in the country. Children with this disease have renal Fanconi syndrome, poor growth, multisystem involvement with cystine crystals causing deterioration of many organs, and renal failure by 10 years of age. Pre-renal transplant patients have been treated with cysteamine since 1978, and a national collaborative study reported by the Section demonstrated proven efficacy of chronic oral cysteamine in retarding renal deterioration and improving growth. In another clinical trial, cysteamine eyedrops were shown to clear crystals from the corneas of two young cystinotic children within 6 months. Another 13-year-old boy had a clear cornea 14 months after a penetrating keratoplasty was performed for intractable pain. His cultured corneal cells accumulated cystine, which was depleted by cysteamine treatment, and his eye tissue exhibited class II antigens, an indication of the inflammatory stimulus issued by cystine storage.

Complications of cystinosis in post-renal transplant patients were also described. Neurological, muscular, ophthalmic, and pancreatic involvement were all reported, and cysteamine therapy is now being offered these patients in an attempt to prevent some of their complications.

One patient with methionine adenosyltransferase deficiency was investigated by methyl and sulfur balance studies. Through this one individual, it was demonstrated that S-adenosylmethionine regulates homocysteine partitioning in man between remethylation to methionine and catabolism to inorganic sulfate.

In an ongoing study, carnitine-deficient Fanconi syndrome patients are being treated with oral L-carnitine. Muscle biopsies have revealed evidence that, in some patients, carnitine deficiency can be effectively treated by repletion therapy.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00131-13 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Biochemical Genetics

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	William A. Gahl	Head	HGB, NICHD
Others:	Isa Bernardini	Technician	HGB, NICHD
	Gregory Harper	Visiting Fellow	HGB, NICHD
	Martin Renlund	Visiting Scientist	HGB, NICHD
	John Hopwood	Guest Researcher	HGB, NICHD
	Jean Butler	Chemist	HGB, NICHD
	Meagan Adamson	NRSA Fellow	HGB, NICHD
	Hans Anderson	IRTA Fellow	HGB, NICHD

## COOPERATING UNITS (if any)

See Attached

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Human Biochemical Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.2

## PROFESSIONAL:

4.2

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Thirty children with cystinosis contributed data toward a national study demonstrating the efficacy of oral cysteamine therapy in enhancing growth and retarding renal failure. In addition, cysteamine eyedrops proved efficacious in removing cystine crystals from the corneas of young children with cystinosis. Late complications of cystinosis are described, including cerebral atrophy, diabetes mellitus, pancreatic exocrine dysfunction, muscle atrophy with parenchymal crystal accumulation, and ophthalmic involvement. One patient received a successful corneal transplant. Carnitine-deficient individuals with Fanconi syndrome continue to be treated with oral carnitine with some success in normalizing their muscle histology. 2) Sialic acid transport across the lysosomal membrane was shown to be defective not only in Salla disease but also in infantile free sialic acid storage disease fibroblasts. Renal handling of free sialic acid and sialic acid metabolism in sialuria variants have been preliminarily investigated. 3) Lowe (oculocerebrorenal) syndrome fibroblasts manifested normal rates of proteoglycan synthesis and sulfation, but an increased activity of nucleotide pyrophosphatase. An HPLC method for separating proteoglycans was described. 4) The lysosomal transport system for tyrosine and other neutral amino acids, discovered in rat FRTL-5 thyroid cell lysosomes, was shown to be TSH-responsive. 5) Preliminary evidence shows that the FRTL-5 cells contain a lysosomal carrier for MIT, explaining how iodine is salvaged for reutilization by these cells. 6) Sulfur and methyl balance studies on an MAT-deficient patient demonstrated that, in vivo, S-adenosylmethionine regulates the partitioning of homocysteine between degradation to inorganic sulfate and remethylation to methionine.

## Cooperating Units:

F. Tietze, NIDDK  
S. H. Mudd, NIMH  
J. Schneider, University of California at San Diego  
J. Thoene, University of Michigan  
G. Thomas, Johns Hopkins University  
N. Bashan, Beersheva, Israel  
W. Rizzo, Medical College of Virginia  
M. Kaiser-Kupfer, NEI  
H. Levy, Massachusetts General Hospital  
J. Schulman, IVF Institute, Fairfax, Virginia  
B. Wolf, Medical College of Virginia  
J. Hoofnagle, NIDDK  
P. Fox, NIDR  
B. Baum, NIDR  
V. Hascall, NIDR  
M. Dalakas, NINCDS  
P. Backlund, NIMH  
J. Finkelstein, VA Hospital, Washington, D.C.  
B. Fivush, Johns Hopkins Medical Center  
C. Porter, George Washington University Medical Center  
R. Chesney, University of California, Davis  
G. Merriam, NICHD  
A. Tangerman, Nijmegen, The Netherlands  
J. Williams, Univ. Texas, Houston  
J. Fink, NINCDS  
L. Kohn, NIDDK  
E. Grollman, NIDDK  
O. Hurko, Johns Hopkins University  
L. Rome, UCLA  
G. Reed, NICHD  
J.W. Balfe, Toronto  
S. O'Regan, Montreal  
K. Ishak, AFIP  
M. Datiles, NEI  
T. Kuwabara, NEI  
J. Hoeg, NHLBI  
M. Beilstein and P. Whanger, U. Oregon



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00133-10 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Glycogen Storage Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James B. Sidbury Head.

HGB, NICHD

## COOPERATING UNITS (if any)

Pamela Bbye, RD, CC, NIH

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.3

## PROFESSIONAL

0.3

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The study is designed to test the heterogeneous responses of different patients with glucose 6 phosphatase deficiency in hydrolyzing and absorbing glucose when administered raw starches from different sources. When divergent results are obtained, family studies are pursued. Further, the results obtained are supplied to the management of the patients with glucose 6 phosphatase deficiency.

Using cooked and uncooked starches which have been tested *in vivo*, we have measured amylase *in vitro* with cooked and uncooked starches using commercial amylase and patients sera. We did not see the 1:1 correlation of *in vivo* and *in vitro* results expected.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00403-06 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Magnesium Metabolism in Mothers and Neonates

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Joan L. Caddell

Guest Researcher

HGB, NICHD

Others: James B. Sidbury

Head

HGB, NICHD

## COOPERATING UNITS (if any)

Joan Blanchette-Mackie (NIADDK, NIH); Kathleen Snowden and Nathaniel Jackson (Small Animal Section, VR, NIH)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The audiogenic seizure-shock episode of Mg deficiency was studied in weanling rats, with emphasis on pulmonary pathology and on means of aborting the syndrome.

Lungs studied in Mg-deficient weanling rats that were killed immediately after shock revealed pathology compatible with many forms of shock, including the respiratory distress syndrome of the human infant. These included denuded basement membranes of the alveoli, and components of the basement membrane (fibrin, precipitated plasma protein, erythrocytes, and cellular debris) within the alveoli. The lungs were edematous, showed hemorrhage and atelectasis, and were essentially airless.

Lung of Mg-deficient rats that were undisturbed after the seizure-shock episode revealed pathology compatible with that of the sudden infant death syndrome (SIDS). After the acute attack, 28 g animals silently and quickly died, while 35-40 g rats experienced hyperventilation and hyperextension of the limbs and cervical spine that served to expand the lungs. Their lungs were well inflated, showed pleural petechiae, with areas of congestion, atelectasis and only mild to moderate edema with occasional hemorrhage. Most of the alveolar spaces were clean; the pathology was insufficient to explain death.

Studied to abort the audiogenic seizure-shock episode of Mg-deficient weanling rats showed that Mg in high doses resulting in unacceptably high plasma Mg values did not protect from seizure activity. Methylprednisolone in high doses did not fully protect from seizures. The combination of reduced Mg dosage and high methylprednisolone given 15-20 min before auditory stress did protect from seizures. Ibuprofen gave full protection from seizures under similar experimental conditions. A Squibb thromboxane A<sub>2</sub> receptor blocker given 15-20 min before challenge modified the episode and aborted tetany, but did not fully protect; it apparently works with other mediators of shock in the pathogenesis of the episode. This is under study.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00404-05 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Jean DeBrohun Butler Senior Investigator HGB, NICHD

## COOPERATING UNITS (if any)

P. Pentchev, NINCDS; S. Padilla, EPA

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Biochemical Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anomalies in cholesterol metabolism uncovered similar to:
  - a) Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification.
  - b) Niemann-Pick D cells which do not store cholesterol but do show a lack of cholesterol esterification.
2. Studies of cholesterol metabolism in Niemann-Pick C and D, and cystinotic fibroblasts.
3. Characterization of cystinotic cell metallothionein present in a 2-fold excess in cystinotic versus normal fibroblasts.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00405-09 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Structure of the Methionine Initiator tRNA Genes in the Human Genome

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

P.I.: Michael A. Zasloff Head HGB, NICHD

Others: Samuel Adeniyi-Jones Visiting Scientist HGB, NICHD  
Janet A. Tobian Staff Fellow HGB, NICHD  
Pilar de la Pena Visiting Fellow HGB, NICHD  
Anthony Adams Biologist HGB, NICHD

## COOPERATING UNITS (if any)

## LAB BRANCH

Human Genetics Branch

## SECTION

Section on Molecular Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

4.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies continue in the area of RNA transport and processing. The 5' tRNA processing nuclease was fully characterized this past year and found to represent an enzyme of extraordinary size and structure. Studies on mRNA nuclear transport were brought to an initial phase of completion by our discovery of a novel and unexpected role of the promoter of a gene in the transport process. Our studies have demonstrated a particularly crucial role for the TATA homology in the disposition of mRNA between nucleus and cytoplasm of a eukaryotic cell.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00408-04 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Pathophysiology and Treatment of Human Genetics Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Michael A. Zasloff

Head

HGB, NICHD

Others: Joan Marini

Staff Fellow

HGB, NICHD

Kenneth Huttner

Staff Fellow

HGB, NICHD

Anthony Adams

Biologist

HGB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Molecular Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

4.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies at the basic and clinical level continue in several heritable bone disorders. In addition, a novel family of vertebrate anti-microbial peptides isolated from Xenopus skin were characterized. Extensive study of the molecular lesions at the procollagen loci in patients with osteogenesis imperfecta was undertaken to further define the relationship between the defect at the protein level and the clinical phenotype. A novel method was developed for mapping point mutations in mRNA species, utilizing RNA-RNA mismatch. Clinical studies on the basis of growth failure in patients with O.I. have identified deranged expression of growth hormone in several individuals with classical O.I., and hormonal intervention has been initiated. Cloning of the human and bovine bone-specific alkaline phosphatase cDNA has continued. A novel family of anti-microbial peptides were isolated from Xenopus skin and a corresponding cDNA was cloned.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00410-02 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Metabolism in Children with Glycogen Storage Disease, Type I

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James B. Sidbury

Head

HGB, NICHD

## COOPERATING UNITS (if any)

Laboratory of Theoretical and Physical Biology, NICHD (Drs. N. Estaban and A. L. Yergey)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This study was designed to determine the rate of glucose production by the liver in patients with absent glucose 6 phosphatase, deficient glucose 6 phosphatase and deficient translocase I as well as type III glycogenosis. There are reports that the liver of patients with type I glycogenosis produce some glucose. This interpretation was to be tested to determine whether there is a detectable difference in glucose production by the liver of those individuals who have a total absence of glucose 6 phosphatase in contrast with those with a partial defect. Similarly, is there a difference in patients with translocase I defect compared with glucose 6 phosphate defect? Is there a difference in liver glucose production by patients with the translocase I defect who have milder manifestations when compared with the more severely affected?



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00411-02 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Nalmefene, an Endorphin Antagonist, in the Control of Appetite

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James B. Sidbury Head

HGB, NICHD

## COOPERATING UNITS (if any)

Pamela Bbye, RD, CC, NIH

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.2

## PROFESSIONAL

0.2

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study was designed to assess the efficacy of nalmefene--a third generation naloxone in the control of appetite. The initial subjects were Prader-Willi Syndrome patients. The medication was increased stepwise over a three week period to 40 mg per day. The patients were then followed for two months in the outpatient department, receiving nalmefene for two weeks, alternating with placebo for two weeks. A final week in the Clinical Center was used for careful evaluation. No effect of the medication was found on food intake in any of the 5 Prader-Willi syndrome patients. Protocol discontinued.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00909-08 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Fetal Alcohol Syndrome

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

P.I.: Anil B. Mukherjee

Head

HGB, NICHD

Others:

Sondra W. Levin

Guest Researcher

HGB, NICHD

Moon John Kim

Stay-in-School

HGB, NICHD

## COOPERATING UNITS (if any)

M. Evans (Wayne State University, Detroit, MI); B. Cowan (University of Mississippi, Jackson, MS);  
P. Martin (Vanderbilt University, Nashville, TN)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Developmental Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

0.5

## OTHER

0.5

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The search for genetic predisposing factors in developing fetal toxicity of ethanol is continued. We admitted two patients at the Clinical Center with the diagnosis of fetal alcohol syndrome under the clinical protocol 83-CH-228. One additional patient with FAS was also seen at an outpatient clinic of Howard University Hospital. Skin biopsies from two of these patients were obtained and fibroblast cultures were established. These cells were used for transketolase enzyme kinetics. Preliminary results indicate an abnormal  $K_m$  for TPP (2.5  $\mu$ M) for one of these patients while the second patient's  $K_m$  was within normal limits. Three age and sex matched control patients' fibroblasts were also studied and all had normal  $K_m$  for TPP (0.5-1.2  $\mu$ M). A three year review has been completed on this protocol and the ICRS has approved this protocol for three more years because of the difficulty in recruiting patients with FAS.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00910-08 HGB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology, Biochemistry and Physiology of Endogenous Antiinflammatory Proteins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Anil B. Mukherjee Head HGB, NICHD

Others: Lucio Miele Visiting Fellow HGB, NICHD

Antonio Facchiano Visiting Fellow HGB, NICHD

Eleanora Cordella-Miele Guest Researcher HGB, NICHD

## COOPERATING UNITS (if any)

B. Cowan (Univ. Mississippi); Howard Zacur (Johns Hopkins University), N. Dubin (Johns Hopkins); R. Dhanireddy (Georgetown University); Sondra Levin (Walter Reed Army Medical Center)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Developmental Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.50

## PROFESSIONAL:

2.25

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Several phospholipase  $A_2$  ( $PLA_2$ ) inhibitory proteins have recently been suggested to regulate the arachidonate cascade and may act as endogenous antiinflammatory agents in vertebrates. Some of these inhibitors are structurally related and are collectively known as lipocortins. Uteroglobin (UTG) is a potent  $PLA_2$  inhibitor but genetically distinct from lipocortins. Because of their similarity in function (i.e.,  $PLA_2$  inhibition) we compared the structure of these proteins. We found that there is considerable peptide sequence homology between lipocortin I and II and UTG and a striking similarity in the hydropathy profiles of UTG, the corresponding regions of lipocortins and  $PLA_2$ . Based upon these results we have custom synthesized peptides of these homologous regions and tested for their  $PLA_2$  inhibitory activity. We found that these peptides are 1000 times more potent  $PLA_2$  inhibitors than UTG as a whole molecule. The results suggest that these peptides may be the active sites responsible for the  $PLA_2$  inhibitory activity of UTG. When these peptides were tested in vivo they were found to be extremely potent antiinflammatory agents in carragenin-induced inflammation in the rat. Because of its potential medical importance as an antiinflammatory agent, we have initiated and been successful in producing this protein in E. coli by recombinant DNA technology. Recently, we have discovered a human counterpart of this protein.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00912-08 HGB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Regulation and Cellular Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Janice Y. Chou Head HGB, NICHD

Others: Shuichiro Watanabe Visiting Fellow HGB, NICHD  
Yvonne Wan Staff Fellow HGB, NICHD  
Juan L. Jimenez-Molina Visiting Fellow HGB, NICHD  
Yoomi Choe Adjunct Scientist HGB, NICHD  
Adam Sartwell Lab. Aid HGB, NICHD

COOPERATING UNITS (if any)

Drs. I. Sun and F.L. Crane (Purdue Univ., IN); Dr. G. Yeoh (Univ. of Western Australia, Australia);  
Dr. I. Boime (Washington Univ., MO); Dr. L. Levenbook (NIADDK); Dr. W. Hoppner (Universitäts-  
Krankenhaus, Federal Republic of Germany); Dr. B-W Soong (DMNB, NINCDS)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Cellular Differentiation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.5

PROFESSIONAL:

4.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. Hybridization, S1 mapping, and primer extension experiments demonstrated that the 2.2-kb and 1.7-kb AFP mRNAs contain identical sequences from the eighth (G) exon to the 3' end of the 2.2-kb RNA but differ in sequences at the 5' end. The 1.7-kb RNA lacks sequence of the seven 5' exons present in the 2.2 kb RNA. However, this variant RNA contains additional sequences 5' to the G exon.

The ts rat fetal hepatocytes grown at 40°C matured into the adult hepatocytes in the presence of glucocorticoid hormones. This was demonstrated by the inhibition of fetal gene but induction of adult gene expression by glucocorticoids. We have also demonstrated that adult liver produced an AFP mRNA which is indistinguishable from the 1.7 kb AFP mRNA. Glucocorticoids stimulated synthesis of the variant AFP encoded by the 1.7 kb RNA but did not enhance production of the corresponding mRNA.

Expression of tyrosine amino transferase (TAT) gene in the ts adult hepatocyte line was ts and dependent upon the presence of glucocorticoid. cAMP alone was not sufficient to induce TAT gene expression, but it enhanced the induction by the steroid hormone.

cDNAs encoding human placental pregnancy-specific  $\beta$ 1- glycoprotein (PS $\beta$ G) have been isolated and characterized. Two cDNA clones (PSG16 and PSG93) differ only in sequence in the 3' end of the coding region. PSG93 contains an additional 86 bp at the end of the 3' coding region of PSG16. This insertion results in the generation of PS $\beta$ G species of 418 amino acid residues instead of the 416 amino acid residues predicted by PSG16. These cDNA probes were used to study regulation of PS $\beta$ G gene expression in human placental fibroblasts. We found that the PS $\beta$ G synthesized by the fibroblasts was structurally different from placental PS $\beta$ G.



## LABORATORY OF COMPARATIVE ETHOLOGY

- Z01 HD 00054-13 Structural and Behavioral Analysis of Vocal Communication  
in Squirrel Monkeys  
D. Symmes, Ph.D.
- Z01 HD 00062-11 Brain Mechanisms of Vocal Production in Squirrel Monkeys  
J. D. Newman, Ph.D.
- Z01 HD 00702-07 Genetics of Primate Communication  
J. D. Newman, Ph.D.
- Z01 HD 01102-06 Behavioral Correlates of Endocrine Disorders in Children  
Robert P. Klein, Ph.D.
- Z01 HD 01104-05 An Observational Study of Parent-Infant Interaction  
in a Family Context  
Frank A. Pederson, Ph.D.
- Z01 HD 01106-04 Developmental Continuity of Individual Differences  
in Rhesus Monkey Reactivity  
Stephen J. Suomi, Ph.D.
- Z01 HD 01107-04 Adaptation of Laboratory Reared Monkeys to Field  
Environments  
Stephen J. Suomi, Ph.D.
- Z01 HD 01108-03 Comparative Studies of Play Behavior  
Maxeen Biben, Ph.D.
- Z01 HD 01110-02 Intuitive Parenting of Infants in Comparative Perspectives  
Stephen J. Suomi, Ph.D.
- Z01 HD 01111-02 Factors Affecting Nurturant Behavior Toward Infants  
Frank A. Pedersen, Ph.D.
- Z01 HD 01112-01 Development of Individual Differences in Social and  
Cognitive Competence  
Michael E. Lamb, Ph.D.
- Z01 HD 01113-01 Antecedents, Correlates, and Consequences of Adolescent  
Pregnancy and Parenthood  
Michael E. Lamb, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Laboratory of Comparative Ethology

The Laboratory of Comparative Ethology (LCE) carries out a program of research directed toward the study of behavioral and biological development in humans and in nonhuman primates. The influences of both genetic and environmental factors, and their interactions, on developmental processes are explored in a comparative approach in order to determine the origins, ontogeny, and evolution of various behavioral phenotypes. Longitudinal designs are employed in most developmental studies in order to address issues of ontogenic continuity vs. change, and both behavioral and biological measures reflecting multiple levels of analysis are collected concomitantly in these studies. A major emphasis is placed on characterizing and understanding normative patterns of biobehavioral development so that deviant patterns can then be readily recognized and their consequences evaluated with respect to established norms. Experimental results in nonhuman primates are correlated with the results of longitudinal studies of human infants and their families as well as with results obtained by various neuroscience techniques.

The LCE consists of four sections. The Comparative Behavioral Genetics section, headed by Dr. Suomi, investigates various processes underlying biological and behavioral development in selected nonhuman primate species by focusing on interactions between genetic and environmental factors that affect the course of an individual's ontogeny. Parallel patterns are then examined in human subjects. Within the Section, the Unit on Neuroethology, headed by Dr. Newman, uses neuroscience techniques to study brain mechanisms involved in the production of various types of primate vocalizations by squirrel monkeys and to examine subtle acoustical differences in characteristic calls between closely related New World primate species. The Brain, Behavior, and Communication Section, headed by Dr. Symmes, studies the use of vocal signals by group-living squirrel monkeys, both in terms of the acoustical properties of the signals and in terms of their information content for group members. Parallel acoustical analyses are conducted on selected vocal patterns of other primate species, including humans. The recently reorganized Child and Family Research Section, now headed by Dr. Bornstein, examines perceptual, cognitive, and dispositional development in human infants and children, with special emphasis on studying the relationships among early attentional processes, social stimulation from caretakers, and subsequent cognitive capabilities. Finally, the newly created Section on Social and Emotional Development, headed by Dr. Lamb, studies the effects of different types of caretaking arrangements on infant and toddler social and emotional development and cognitive competence. Special attention is given to longitudinal approaches that involve cross-cultural comparisons and those examining nonnormative samples of both parents and infants, in an effort to disentangle genetic and environmental effects and

interactions that shape individual human social and emotional development.

During the past year the LCE underwent major expansion in terms of administrative organization, professional staff, and research space and facilities. A new section, the Section on Social and Emotional Development, was created de novo, while the Child and Family Research Section was re-organized in terms of constituent Units and a new Section Chief was recruited. Dr. Marc H. Bornstein, formerly Professor of Psychology and Human Development and Adjunct Professor of Psychiatry at New York University, joined the LCE in September, 1987 to assume the position of Chief, Child and Family Research Section and to oversee the Section's re-organization. Dr. Bornstein has achieved international recognition for his previous research in the area of human infant perceptual and cognitive development, and he plans to continue and expand on these efforts in the LCE. Dr. Michael E. Lamb, formerly Professor of Psychology and Research Professor of Pediatrics and Psychiatry at the University of Utah, was successfully recruited to develop the new Section on Social and Emotional Development within the LCE. Dr. Lamb, who is one of the world's authorities on social and emotional development in children, joined the LCE in June, 1987. In addition, two new institutional NRSA trainees, two individual NRSA postdoctoral fellows, a Fogarty visiting fellow, and two new IRTA postdoctoral fellows began working in the LCE during the past year.

A major construction project, the renovation of Building 112 to create a state-of-the-art nonhuman primate research facility at the National Institutes of Health Animal Center (NIHAC), was completed late in the fiscal year and was duly occupied by LCE staff and subjects. This renovated building, featuring indoor-outdoor group pens as the standard unit for housing macaque subjects, greatly expands the LCE's research capabilities at the NIHAC site and will now permit the relocation of the LCE's research activities currently maintained at the University of Wisconsin Primate Laboratory to the new facilities at the NIHAC. In addition, the final architectural plans were completed for a new joint NICHD-NIMH facility at the NIHAC (Building 110A) designed explicitly in part for state-of-the-art indoor-outdoor housing of New World primates in the LCE research program. Actual construction of Building 110A is scheduled to begin in the fall of 1987. Also, preliminary plans for 4 new multi-acre enclosures for macaques and a year-round shelter facility serving each enclosure were completed and a more complete feasibility study initiated by DES. Space appropriate for housing the research activities of the new Section on Social and Emotional Development was obtained off-campus (in the Boy Scouts of America National Headquarters building). Finally, minor remodeling of the LCE's Building 31 human developmental laboratory was begun in order to accommodate the demands of Dr. Bornstein's research program. A summary of the major research activities of each of the LCE's sections during the past year follows.

## Comparative Behavior Genetics Section (CBGS)

A major research focus of the CBGS is the determination of genetic and environmental factors that individually or in concert shape the course of rhesus monkey biobehavioral development. This past year major advances were achieved in demonstrating the heritability of specific behavioral and physiological responses to minor environmental challenges and in characterizing the nature of early environmental influences on subsequent responses to challenge. Heritability of individual differences in response to challenge was explored in two studies, the first of which compared peer-reared paternal half-siblings (same father, different mothers) between sires in terms of behavioral, neuroendocrine, and immunological responses to short-term peer separation at 6 months of age, and in relative position in the dominance hierarchy in peer groups assembled subsequent to the separations. Clear-cut differences on many of these measures emerged between sets of paternal half-siblings: offspring of certain breeding colony males consistently exhibited higher levels of plasma ACTH and behavioral disturbance, and diminished responsiveness to mitogen challenge, than offspring of other breeding colony males. These findings are of special interest regarding the relative heritability of these different patterns of response because the paternal half-siblings under comparison were born over a span of 3 years, they were reared in different social groups from one another, and they never saw, let alone interacted with, their biological fathers at any time during the study.

The second experiment examined the reaction to mild environmental challenge by the above-described breeding colony males themselves. Each male was removed from its home cage and housed in a novel room for a 2-day period; a second brief (2-hr.) period of removal from the home cage took place several weeks later. Behavioral, neuroendocrine, and immunological data were collected prior to, during, and following the periods of home cage removal. Striking differences in response to these mild challenges between individual males were found for a variety of measures, including self-directed behavior, activity levels, absolute levels of lymphocytes, and lymphocyte-neutrophil ratios (the neuroendocrine data are still being analyzed), although such differences were diminished during baseline and post-challenge periods. More specifically, those males producing offspring who displayed extreme reactions to social separations clearly exhibited the most extreme reactions to challenge, whereas those breeding colony males producing offspring who displayed only mild reactions to separation likewise tended to exhibit very mild behavioral and physiological responses to brief removal from their respective home cages. Thus, these breeding colony males and their respective first-generational offspring displayed similar patterns of response to challenge, despite the fact that there was no opportunity for the offspring to "learn" or be otherwise influenced by their fathers through directed interactions. These findings represent perhaps the first unambiguous demonstration of intergenerational



consistency in patterns of individual differences, in the clear absence of any opportunity for intergenerational information transfer, in any behavioral system in any nonhuman primate species.

Additional data from ongoing longitudinal studies of several other groups of rhesus monkeys again confirmed that such individual differences in response to challenge, whatever their basis might be, were clearly stable over periods of major developmental change. For example, significant concordance between values obtained from like-reared monkeys during challenges at 6 and 18 months of age were found for measures of self-directed and huddling behavior, plasma cortisol and ACTH, and CSF levels of NE, MHPG (a NE metabolite), and HVA (a dopamine metabolite). In another study, short-term stability of several immune system responses, including immunoglobulin levels of IgG and IgM, absolute levels of lymphocytes, and lymphocyte-neutrophil ratios, was observed in 6-month-old peer-reared subjects. Stability of individual differences in heart rate and vagal tone during repeated 1-hour introductions to a novel social playroom was found in a third study of infant and juvenile rhesus monkeys. Thus, individual differences in biological response to environmental challenge that appear to be highly heritable display substantial stability, both in the short and long term, paralleling previous findings of stability of behavioral response to comparable environmental challenges.

A final set of findings regarding individual differences in rhesus monkey response to challenge came from a study initiated in collaboration with the Caribbean Primate Research Center, in which members of a long-standing troop of wild-born rhesus monkeys, which in 1984 had been captured and moved from its original habitat on the island of Cayo Santiago to a 3-acre enclosure at the Caribbean Primate Research Center's facilities at Sabana Seca, Puerto Rico, were "screened" for relative reactivity during the time of annual capture by staff veterinarians for tetanus shots, tuberculosis tests, and morphometric measurements. This routine veterinary procedure afforded the opportunity to obtain blood samples and behavioral observations from each member of the wild-born troop following capture. Blood samples were assayed for levels of plasma cortisol and ACTH, as well as to provide the basis for identification of paternity. Preliminary analyses of the cortisol, ACTH, and behavioral data have clearly revealed a range of individual differences on these measures that are quite comparable to values obtained from the LCE's captive-born colony. Moreover, there are clear age, sex, and matriline differences on these measures that match those reported in our previous studies utilizing the LCE colony. Thus, to date the results of this study have revealed considerable generality between these semi-field data and a large body of findings from the laboratory. Questions regarding the relative heritability of the patterns of individual differences in response to challenge observed in the Puerto Rican monkeys await the results of the paternity analyses.

Heritability of a different sort, that of basic species-characteristic patterns of behavioral development and social organization, con-

tinued to be explored in a long-term study of laboratory-reared rhesus monkeys' adaptation to free-ranging outdoor settings modelled after field environments. A special focus of this research has been on status changes of males as they pass through puberty, resulting ultimately in emigration (actually, in most cases, expulsion) from their natal troop. Details of this process, occurring spontaneously in the laboratory-reared group as it does in feral troops, were recorded on a case-by-case basis. A parallel longitudinal study of male peripheralization was initiated on the free-ranging wild monkey troops living on Cayo Santiago in another collaborative study with the Caribbean Primate Research Center. This study has been designed to track prospectively the process of adolescent natal troop emigration through longitudinal study of selected juveniles in representative troops on the island. In addition to behavioral observations conducted throughout the year, annual measurements of physical growth and maturation, hormonal profiles, and psychophysiological reactivity are being obtained from these juvenile and adolescent males during their annual capture by CPRC veterinary staff for tetanus shots and TB testing. Findings from this observational study will be used to develop the basis for simulating male transfer in the LCE's proposed new outdoor enclosures at the NIHAC. The significance of these studies lies in the capability to identify processes underlying possible differential adaptive fitness of bio-behavioral phenotypes under controlled social-environmental conditions based directly on observations of the phenomenon in field settings.

Another major area of study in the CBGS this past year examined the contribution of differential rearing conditions to individual differences among rhesus monkey juveniles and preadolescents of known genetic pedigree by comparing responses to challenge between subjects either reared by their mothers for their first 6 months of life or raised in peer groups following nursery rearing; after 6 months all subjects entered new peer groups. Data analyzed to date have revealed that there were few differences in behavior or physiology when subjects remained in their stable social groups. Indeed, in these mixed groups nursery-reared subjects were as likely to be at the top of the dominance hierarchies as were mother-reared subjects. However, social separation produced substantially more behavioral disruption, in terms of higher levels of abnormal, self-directed behavior, greater passivity, and less environmental exploration at both 6 and 18 months, in nursery-reared subjects than in mother-reared ones. Plasma cortisol and ACTH levels were also significantly higher in nursery-reared subjects, but only at 6 months of age. Levels of CSF MHPG were higher at both ages and levels of CSF 5-HIAA were lower at 18 months (but not at 6 months) in nursery-reared subjects. Immune system responsiveness did not differentiate among the 2 rearing conditions at 6 months (the 18-month data await analysis).

Collaborative studies with LCS, NIMH and the LCS, NIAAA involving older monkeys raised in differential social environments but subsequently living in mixed groups also revealed both behavioral and

physiological differences following specific pharmacological challenges. Nevertheless, within each rearing group individual differences in response to those challenges remained largely stable, as in the previously described studies. These results demonstrate that it is possible to characterize the nature of genetic-early rearing environmental interactions influencing the development of specific biobehavioral systems in rhesus monkey subjects.

The effects of differential early rearing environments were additionally investigated in a long-term follow-up of infants differing in risk for developing severe reactions to challenge who had been foster-reared by multiparous females chosen on the basis of their treatment of previous offspring. High-reactive infants cross-fostered by nurturant females continued to dominate their respective peer groups long after all associations with their foster mothers had ended, in comparison with high-reactive infants cross-fostered with "anxious" females and all low-reactive infants regardless of the type of foster mothering they had received. Such findings again demonstrate that individual patterns of developmental change are clearly the product of genetic-environmental interactions that can now be specified in considerable detail and that afford the opportunity for rather precise predictions of long-term developmental outcomes.

A final set of studies investigating the consequences of differential early experiences for subsequent biobehavioral development focused on experimental "enrichment" of early rearing environments for infants reared either with their mothers or in the nursery followed by peer group living. For nursery-reared infants, environmental enrichment involved exposure to a cloth-covered surrogate mounted on springs, facilitating vestibular and kinesthetic stimulation, while for mother-reared infants, environmental enrichment involved rearing within a nuclear family social environment, with exposure to fathers, siblings, peers, and unrelated adults, as compared to rearing in single cages containing only the infant's biological mother. Comparison of neonatal test scores between enriched and nonenriched infants revealed relatively few significant differences within either rearing group during the first month of life. In contrast, there were major differences in performance on cognitive tests at 8 months of age, with both enriched mother-reared and enriched nursery-reared subjects achieving significantly superior scores than their counterparts reared in each of the "standard" conditions. These findings thus support the "delay" hypothesis, i.e., that early environmental enrichment can have significant positive consequences for subsequent cognitive development, even if there are few obvious effects apparent during the initial period of enrichment.

Age-based developmental norms for tests of neonatal and infant behavioral capabilities and characteristics that have been established in two large normative samples are also being employed in evaluations of neonatal development in other selected groups of infants. In one collaborative study with Dr. M. Michejda, hydrocephalic rhesus sub-



monkey infants subjected to experimental interuterine shunt at 120 days gestation are being assessed on the neonatal battery of tests to determine the neurological and cognitive effects of the prenatal surgical intervention (previous work by Dr. Michejda and others has demonstrated that untreated hydrocephalic rhesus monkey infants invariably are severely retarded and rarely survive the first week of life). The two experimental infants tested to date (one currently 2 months old, the other 2 weeks) have thus far shown species-normative patterns of scores on virtually all test items, strongly suggesting that the prenatal shunting procedure is highly effective, at least for these measures. The rhesus monkey infant developmental norms are also being utilized as a basis for comparison of biobehavioral development in nursery-reared chimpanzees (Pan troglodytes) and in hand-reared capuchin monkeys (Cebus apella), in collaborative studies with Dr. Nadler of the Yerkes Regional Primate Research Center and Dr. Visalberghi of the Istituto di Psicologia, CNR. Preliminary data from both studies are extremely promising in terms of providing a useful basis for cross-species comparisons of developmental processes.

Unit on Neuroethology: During the past year, the the Unit on Neuroethology, directed by Dr. Newman, initiated comparative studies of acoustical features of New World primates both between species (common marmosets, pigmy marmosets, and squirrel monkeys) and within genus (squirrel monkeys from different geographical areas). Findings from these new studies include the following: (1) the isolation calls of pygmy marmosets over their first 8 weeks of life are distinguishable from those of mature individuals by virtue of the presence of numerous sounds with pronounced frequency modulation ("trills" and "phee notes"), in addition to the species-typical properties of repeated strings of more stereotyped notes with a characteristic frequency upsweep ("J-notes"); (2) young adults of the common marmoset, Callithrix jacchus, are robust sources of isolation calls when visually or acoustically separated from familiar colony members, producing long, loud whistles with individually distinctive structures that show significant and regular changes in certain parameters as time of separation increases; and (3) the squirrel monkeys of Costa Rica, which represent remnant populations with a long history of geographical isolation from the principal range of the genus in South America, demonstrate the same strong correlation between facial subtype and the acoustic characteristics of their speciestypical isolation call, the Isolation Peep, as do South American squirrel monkeys.

The other major focus of the Unit on Neuroethology is on investigation of the effect of neurological and pharmacological interventions on the incidence and structure of squirrel monkey vocalizations emitted spontaneously in naturalistic settings that can be reliably modelled under laboratory conditions. This past year studies investigating the possible involvement of an endogenous opiate-mediated process in the production of isolation calls yielded significant findings of considerable theoretical importance. Build-



ing on previous results indicating that moderate doses of morphine blocked the production of isolation calls but were reversible by pretreatment with the opiate receptor antagonist naloxone, it was found that naloxone treatment by itself enhanced the production of isolation calls in dose-dependent fashion up to 1.6 mg/kg, after which there was a decrease to or below control values. In a second study performed in collaboration with Dr. Harris, the effects of combining naloxone administration with the alpha 2-adrenergic receptor antagonist yohimbine (which administered by itself also enhances production of isolation calls in dose-dependent fashion) were studied. It was found that the two drugs did not have synergistic effects but instead appeared to be influencing isolation call production through different mechanisms, a finding of considerable theoretical interest and clinical relevance in that it is consistent with the hypothesis that manifestations of separation anxiety (which in humans occurs primarily in children) are fundamentally different than those of generalized anxiety (primarily a disorder of adults). Related studies carried out this past year investigated the effects of other compounds and procedures on the production of species-normative vocal patterns. The long-term objective of these studies is to characterize the nature of neurochemical control of vocal patterns emitted in response to species-normative environmental events.

#### Brain, Behavior, and Communication Section (BBCS)

This section, headed by Dr. Symmes, investigates the use of vocal signals by squirrel monkeys in complex social contexts, focusing on both the acoustical features of the vocalizations and their information content for different group members. Results are then utilized to characterize the nature and possible functions of the specific activities that are displayed concomitant with specific vocal signal. One major research project completed during the past year has involved an analysis of the effects of social play in squirrel monkeys on restricting partner choice. Much of the theoretical treatment of play, a behavior that is widespread in mammalian species and is thought to be important in human development, has focused on the delayed benefits in socialization which may accrue from frequent, normal exposure early in life. In order to reveal these supposed delayed benefits various investigators have tried to alter or inhibit play in animal models but have generated ambiguous results because more than play was eliminated; for example, raising monkeys alone, a strategy used by many previous investigators, deprives the monkeys of nonplay social contact, and produces behavioral outcomes which are suggestive of prolonged depression and serious psychological damage. Dr. Biben's studies have addressed more subtle aspect of play in animals, including the occurrence of role reversal as training for social flexibility. These new data on restricted partner choice demonstrate that the play experience can be manipulated without unwanted generalized effects, and they provide a promising model for conclusive demonstration of the long-sought delayed benefits, and for insights into developmental organization.

The computer based sound analysis system developed by Dr. Symmes (with consultation with BEIB) is proving to be a major asset in the BBCS laboratory, permitting very efficient methodology in what has been historically a labor-intensive area. Several new studies are under way or were initiated late last year utilizing the model language system of monkeys. These studies have already yielded significant findings regarding both structural and temporal rules governing vocal exchanges between squirrel monkeys, permitting the development of an infrahuman primate model of "conversation." The present work focuses on vocal development as assessed in interchanges between squirrel monkey infants and their mothers.

Collaborative work with Drs. Hanus and Mechtilde Papousek has been of great mutual benefit, and the view of these investigators that a melodic mode of communication exists (probably with a genetic basis) and is employed by human mothers and fathers in interacting with the prelinguistic infant is supported by recent cross-cultural studies of native Chinese and English speaking mothers. The data for these studies were largely processed on the BBCS sound analysis system. The model provided by this collaborative enterprise is being actively examined at the animal level.

#### Child and Family Research section (CFRS)

The Child and Family Research Section has a long tradition of investigating cognitive, emotional, and psychosocial development in human infants and children, with a special emphasis on determining the consequences of early family experience on these developmental processes. This past year several studies directed by Dr. Pedersen examined the consequences of specific experiences, both short- and long-term, on these processes. One study focused on effects of maternal workforce participation on patterns of mother-toddler play in laboratory settings and on child compliance behavior in home environments. A second set of studies examined father-infant interactions in the context of presence or absence of the mother in the ongoing interactive setting and as a function of the extent of the father's previous caretaking experience with the child. Findings from these studies have supported the hypothesis that, at least in highly educated middle-class samples of first-born children, maternal care is generally enhanced by reducing long periods of time spent with the infant in solitary contexts, while paternal care may be enhanced by provision for repeated periods of caretaking responsibility without the physical presence of the mother.

A third set of studies examined in greater detail the development of parents' nurturant responses to infants. One experiment carried out by Dr. Berman investigated possible differences between mothers and fathers in communication of expectations in their play behavior in male vs. female infants. A second experiment, conducted by Dr. Kestermann, examined the consequences of a prenatal intervention for first-time expectant mothers designed to reduce their anxiety toward the upcoming birth, to heighten their sensitivity to different

arousal states in infants, and to facilitate appropriate responses to behavioral signals emitted by infants. Anxiety surrounding the anticipated birth of an infant was also investigated in two other studies, the first comparing expectant parents who had experienced previous pregnancy loss (miscarriage, stillbirth, or neonatal death) with first-time expectant parents, and the second investigating possible relationships between the emotional states of mothers during their pregnancy and both postpartum affective state and sensitivity to infant behaviors, especially vocal patterns. Dr. Theut has served as PI for the first study, while Drs. Pedersen, Bryan, and Huffman are carrying out the second study. Data collection has been completed in all of these studies except the last one, for which it is currently underway.

In September 1987, Dr. Bornstein joined the CFRS as the new Section Chief, bringing with him a program of research that will expand considerably the scope of investigations of relationships between infant characteristics, parental care in various settings, and developmental outcomes on a variety of measures of cognitive and social competence. This expanded program of research will place increasing emphasis on studying the actual processes involved in human biobehavioral development, and it will utilize a wider range of subject populations, including cross-cultural samples, in these investigations than has generally been the case to date.

#### Section of Social and Emotional Development (SSED)

The Section on Social and Emotional Development is a new addition to the LCE, having been created this past year, with the Section Chief, Dr. Lamb, joining the LCE in June, 1987. The SSED is still in the process of being organized and moving into its new headquarters in the Boy Scouts of America National Headquarters building, which is immediately adjacent to the NIH Bethesda campus. Nevertheless, Dr. Lamb has been able to continue two major research projects initiated during his previous tenure at the University of Utah. The first project has been examining the effects of center-based day care, family-based day care, and home care in a longitudinal sample of 140 children in Goteborg, Sweden initiated when the children were 16 months of age, with annual follow-up observations of the children completed to date for a 2-year period. Results to date have shown that while the general type of child care has no differentiated impact on social and emotional characteristics of the infants, it has been found that quality of home care, infant temperament, and perceived social support of the parents have been shown to make significant contributions to individual differences among the children throughout the 2-year follow-up, while quality of alternative care have had demonstrable effects in the second, but not the first, year of follow-up observations.

The second project being continued by Dr. Lamb in his new position involves analyses of data from two large nationally-representative samples: the National Longitudinal Study of Youth (NLSY) and the Census Bureau's Current Population Surveys (CPS). Analyses current-



ly underway have been designed to explore the conduct disorders, judicial histories, and behavioral problems of adolescent mothers in the NLSY sample. The goal is to confirm the indication, based on a smaller sample previously collected in Utah, that both adolescent mothers and fathers have a history of diverse conduct disorders, of which pregnancy is but one symptom. Such findings would illustrate the extent to which the multiproblem nature of adolescent parenthood defies simplistic intervention efforts and speaks to the need for a clear and comprehensive understanding of the context of adolescent pregnancy. Other ongoing research using the CPS dataset is designed to examine aspects of the relationship between men and women with varying intracouple age differences and with varying ages at the time of marriage. In October 1987, data will become available on 5000 children born to participants in the NLSY. This will permit analysis focused on the links between styles of parental and filial adjustment to adolescent pregnancy and parenthood.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00054-13 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Structural and behavioral analysis of vocal communication in squirrel monkeys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Symmes Head LCE, NICHD

Other: M. Biben	Senior Staff Fellow	LCE, NICHD
P. Goedeking	Visiting Fellow	LCE, NICHD
D. Bernhards	Bio. Lab. Technician	LCE, NICHD
G. Hetzel	Animal Caretaker	LCE, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Section on Brain, Behavior, and Communication

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.6

## PROFESSIONAL

1.8

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued the study of squirrel monkey calls used in social contexts characterized by quiet affiliative behaviors and lack of internal aggression or external threat. Our program of research on this model of "conversation" in infrahuman primates has yielded significant findings regarding both structural and temporal rules governing vocal exchanges. Results have been published and presented at workshop conferences with excellent professional feedback.

An initiative begun last year concerned with the development of vocal behavior in squirrel monkeys is being pursued vigorously. The essential requirement for this work is a successful breeding program, and that is available in our facilities at Poolesville. Our seasonal outdoor habitats have been very productive in this respect. In this long term study we plan to track vocal development, focusing initially on interactions between mother and infant during the dependency period. Early work has identified a call used in exchanges between mothers or other adult females and infants, which has been poorly described previously and not characterized acoustically.

We have also carried out a study with P. Goedeking (Visiting Fellow) on the spontaneous increase in vocalization rate (called "chorusing" by some workers) heard when lights go out in the evening. This behavior (frequently noted but never studied in squirrel monkeys) is a laboratory model of a natural phenomenon, the significance of which may lie in detailed acoustic changes as visual contact with group members is lost or sharply reduced. This project, as with all our current work, is made possible by the sophisticated sound analysis system now available in our laboratory.

Collaborative research with Drs. Hanus and Mechthild Papousek involving the quantification of acoustic structure in vocal interactions between human mothers and preverbal infants has continued productively and is reported more fully elsewhere.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Vocal Production in Squirrel Monkeys

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. D. Newman Research Physiologist LCE, NICHD

Other: G. Hetzel Animal Caretaker LCE, NICHD

## COOPERATING UNITS (if any)

E. Settle, Bio. Lab. Technician, LCS, NIMH  
LCS, NIMH (T. Insel, P. D. MacLean); BPB, NIMH (J. R. Glowa); LN, NIMH  
(J. Bachevalier); Johns Hopkins School of Medicine (J. C. Harris)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Comparative Behavioral Genetics

## INSTITUTE AND LOCATION

NICHD, NIH

## TOTAL MAN-YEARS

0.7

## PROFESSIONAL:

0.5

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the effect of neurological and pharmacological interventions on the incidence and structure of squirrel monkey vocalizations emitted during standardized conditions in the laboratory. Current work focuses on mechanisms underlying expression of the isolation call, a characteristic vocalization used for reestablishing contact between an infant and its caregiver, and between adult social partners. Previous reports have documented the critical role of mediofrontal limbic cortex in expression of spontaneous isolation calls in squirrel monkeys, and the ability of chemicals selective for specific receptor subtypes (opiatergic mu receptor; alpha-2 adrenergic) to modulate the rate of production of this vocalization. New findings this year are: (1) subadult rhesus macaques with bilateral ablations of the amygdala continue to emit the species-typical isolation call, but the acoustic structure of the calls of these monkeys is abnormal, relative to age-matched controls, in exhibiting a less pronounced peak frequency in the time course of the fundamental, suggestive of reduced affective tone; (2) dose-response relations for isolation call production have been determined for the opiate antagonist naloxone over a dose range of 0.4-3.2 mg/kg, administered i.m. in adult squirrel monkeys; (3) the combined administration of naloxone and yohimbine (a specific alpha-2 adrenergic receptor antagonist) does not enhance isolation call rate, but results in a significant increase in the twitter call in separated squirrel monkeys, suggesting that under certain conditions, drugs with known anxiogenic properties may enhance behavior associated with positive affect; (4) pentobarbital sodium in sedating doses is effective in greatly enhancing production of the isolation call in adult squirrel monkeys; or (5) studies of the pharmacology of another squirrel monkey vocalization, the alarm call, suggest a role of the benzodiazepine receptor in the neurochemical control of the alarm reaction.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00702-07 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Genetics of Primate Communication

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. D. Newman	Research Physiologist	LCE, NICHD
Other:	S. Boinski	NRSA Fellow	LCE, NICHD
	P. Goodeking	Visiting Fellow	LCE, NICHD
	Y. Bryan	Visiting Fellow	LCE, NICHD
	G. Hetzel	Animal Caretaker	LCE, NICHD

## COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH  
E. Settle, Bio. Lab. Technician, LCS, NIMH

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Comparative Behavioral Genetics

## INSTITUTE AND LOCATION

NICHD, NIH

## TOTAL MAN-YEARS

2.1

## PROFESSIONAL:

1.9

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the role of genetic factors in primate vocal development. Current work focuses on the genetic and environmental factors influencing expression of the isolation call, a characteristic vocalization used for reestablishing contact between an infant and its caregiver, and between adult social partners. Previous reports have documented the importance of inherited factors in determining the detailed frequency vs. time pattern of the squirrel monkey isolation call, and that the infant isolation calls of a wide range of primate species are distinguishable by species-specific details but share an overall pattern of slowly modulated tonality. New findings this year are: (1) the isolation calls of pygmy marmosets over their first 8 weeks of life are distinguishable from those of mature individuals by virtue of the presence of numerous sounds with pronounced frequency modulation ("trills" and "phee notes"), in addition to the species-typical properties of repeated strings of more stereotyped notes with a characteristic frequency upsweep ("J-notes"); (2) young adults of the common marmoset, Callithrix jacchus, are robust sources of isolation calls when visually or acoustically separated from familiar colony members, producing long, loud whistles with individually distinctive structure which show significant and regular changes in certain parameters as time of separation increases; and (3) the squirrel monkeys of Costa Rica, which represent remnant populations with a long history of geographical isolation from the principal range of the genus in South America, demonstrate the same strong correlation between facial subtype and the acoustic characteristics of their species-typical isolation call, the Isolation Peep, as do South American monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01102-06 LCE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Behavioral Correlates of Endocrine Disorders in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. P. Klein Senior Research Investigator LCE, NICHD

Other: N. F. Gist Research Psychologist LCE, NICHD

M. Fivel Research Pscyhologist LCE, NICHD

COOPERATING UNITS (if any)

Susan Rose, M.D., Medical Staff Fellow, DEB, NICHD, Developmental Endocrinology Branch, NICHD; Laboratory of Developmental Psychology, NIMH; Child Studies Center, University of Maryland; Division of Endocrinology, Children's Hospital Medical Center; University of Minnesota Medical School (Sonis)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.6

PROFESSIONAL

0

OTHER

0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project encompasses a series of studies examining the behavioral correlates of endocrine disorders in young patients, including children with precocious puberty, Turner's syndrome, and growth hormone deficiency. A first objective was to determine whether these children are at risk for problems in psychosocial adjustment. In a sample of children with precocious puberty, we reported that these children do, in fact, show an above-normal incidence of a variety of adjustment problems. A current objective is to ascertain the factor(s) responsible for this finding. Analyses during the past fiscal year have focused on the combined influence of age of onset, duration of symptoms before treatment was begun, and pretreatment medical status as indicated by bone age, relative height, pubic hair stage and the levels of gonadotropins and sex steroids. In general these factors had a synergistic effect, i.e., their influence was stronger when taking into account the other factors than when looking at their influence individually. This project has been terminated.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01104-05 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An Observational Study of Parent-Infant Interaction in a Family Context

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: F. A. Pedersen Head LCE, NICHD

Other:	R. L. Cain	Research Psychologist	LCE, NICHD
	J. D. Suwalsky	Research Psychologist	LCE, NICHD
	N. F. Gist	Research Psychologist	LCE, NICHD
	M. Fivel	Research Psychologist	LCE, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Child and Family Research Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.6

## PROFESSIONAL:

0.5

## OTHER:

2.1

## CHECK APPROPRIATE BOX(ES)

- |  |  |                                      |
|--|--|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors                   |  |                                      |
| <input checked="" type="checkbox"/> (a2) Interviews    |  |                                      |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project currently encompasses four areas of investigation based on several different samples with a total of approximately 200 families as participants. All studies were conducted with middle class families and first born infants. The focal period of is from early infancy through the first two and a half years of life. Procedures vary with each sample, but include observations of mother-infant and father-infant interaction in the natural home environment, structured interactions in the laboratory, interviews, and questionnaires. The first area of inquiry concerns the effects of maternal workforce participation on the child's early experiences. Structured laboratory observations of mother-toddler play were conducted to test hypotheses related to previous findings that when negative consequences have been reported for children of employed mothers, these tend to occur for males. A second inquiry focused upon the antecedents of a secure mother-infant attachment relationship; the social context in which mother-infant interaction was observed was found to influence the predictability of the subsequent mother-infant attachment relationship. A third area of inquiry is concerned with the father's role in the family. Follow-up observations of father-infant interaction at 1 year of age were analyzed for two groups of men who in the year preceding the observations had contrasting amounts of experience with their infants in contexts separate from the mother. The fourth area of inquiry concerns 3-person interactions, the mutual regulation of visual, vocal, proximity, and contact behavior of mothers, fathers, and infants. Patterns of behavior are being examined that vary depending upon the parents' psychological accessibility to the child and their degree of verbal engagement with one another.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01106-04 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.:	S. J. Suomi	Head	LCE, NICHD
Other:	K. L. Rasmussen	IRTA Fellow	LCE, NICHD
	C. E. Eisele	Research Psychologist	LCE, NICHD
	J. M. Scanlan	Research Psychologist	LCE, NICHD
	M. Champoux	Research Psychologist	LCE, NICHD
	M. Davidson	Research Psychologist	LCE, NICHD
	R. Delizio	Research Psychologist	LCE, NIMH
	K. Thompson	Bio. Lab. Tech.	LCE, NIMH

COOPERATING UNITS (if any) LCS, NIAAA (Linoilla, Higley); CNB, NIMH (Insel); LN NIMH (Murray); Primate Laboratory, Univ. Wisconsin-Madison (Coe, Schneider); Dept. of Obstetrics & Gynecology, Georgetown Univ. Med. Sch. (Michejda); Yerkes Reg. Primate Center (Nadler, Bard); Istituto di Psicologia, CNR (Visalberghi)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Comparative Behavioral Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.4

## PROFESSIONAL:

1.0

## OTHER:

2.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

This project investigates primate biobehavioral development through comparative longitudinal investigations, with special emphasis on studying individual differences among rhesus monkeys in response to mild environmental challenge and on determining their long-term developmental consequences in different physical and social environments. Findings of particular interest from studies completed this past year include the following: (1) Standardized measures of rhesus monkey neonatal reflex, tone, and state change patterns obtained during the first month of life clearly differentiated subjects on the basis of rearing environment and best predictor of optimal performance on cognitive tests administered 6-8 months later. In addition, the cognitive tests revealed positive consequences of early "enrichment" within each rearing environment studied. (2) Ongoing studies of biobehavioral continuity and change from birth to adulthood yielded long-term effects on both behavioral and physiological systems attributable to differential rearing experiences during the first 6 months of life. However, monkeys within each rearing condition were highly stable in terms of individual differences in behavioral, physiological, and immunological measures in response to mild challenge, even in the face of major developmental changes for most of these measures. (3) Comparisons of behavioral, neuroendocrine, and immunological response profiles between breeding colony males and their multiple offspring revealed striking cross-generational similarities in pattern of response, despite the fact that the males never were exposed to these offspring. These findings add to a growing body of evidence suggesting that such patterns are highly heritable. (4) Preliminary data from a new study of response to challenge in a wild rhesus monkey troop revealed patterns of neuroendocrine and behavioral response virtually identical to those reported in laboratory studies of captive-born monkeys, indicating considerable generality of the previous laboratory-based findings and additionally suggesting a viable setting in which to assess the relative adaptive fitness of these different response patterns.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER 201 HD 01107-04 LCE																												
PERIOD COVERED October 1, 1986 to September 30, 1987																														
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Adaptation of Laboratory Reared Monkeys to Field Environments																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">S. J. Suomi</td> <td style="width: 30%;">Head</td> <td style="width: 20%;">LCE, NICHD</td> </tr> <tr> <td colspan="4"> </td> </tr> <tr> <td>Other:</td> <td>K. Rasmussen</td> <td>IRTA Fellow</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>P. O'Neill</td> <td>Research Psychologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>G. DiGregorio</td> <td>Research Psychologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>C. Price</td> <td>Biologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>C. McKenna</td> <td>Psychology Aid</td> <td>LCE, NICHD</td> </tr> </table>			PI:	S. J. Suomi	Head	LCE, NICHD					Other:	K. Rasmussen	IRTA Fellow	LCE, NICHD		P. O'Neill	Research Psychologist	LCE, NICHD		G. DiGregorio	Research Psychologist	LCE, NICHD		C. Price	Biologist	LCE, NICHD		C. McKenna	Psychology Aid	LCE, NICHD
PI:	S. J. Suomi	Head	LCE, NICHD																											
Other:	K. Rasmussen	IRTA Fellow	LCE, NICHD																											
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	G. DiGregorio	Research Psychologist	LCE, NICHD																											
	C. Price	Biologist	LCE, NICHD																											
	C. McKenna	Psychology Aid	LCE, NICHD																											
COOPERATING UNITS (if any)																														
LAB/BRANCH Laboratory of Comparative Ethology																														
SECTION Comparative Behavioral Genetics																														
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20205																														
TOTAL MAN-YEARS 3.3	PROFESSIONAL 0.7	OTHER 2.6																												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided )  <p>             This project involves the longitudinal study of a group of 14-year-old rhesus monkeys and two generations of their progeny, all of whom live year-round in a 5-acre enclosure on the grounds of the NIHAC. The 14-year-old adults were all laboratory born, hand-reared in a nursery, and subsequently put together as a mixed-sex peer group. Despite the fact that none of these now middle-aged monkeys (nor any of their progeny) have had any physical exposure to any other monkeys, since they were first moved outdoors as juveniles they have consistently exhibited the full compliment of species-normative social behavior and group organization reported to date for rhesus monkeys born and living in feral environments. During the past year additional data collected on the main study group continued to document the species-normative patterns of social-emotional development, overall group organization, and seasonally-based patterns of behavioral and hormonal change that to date have characterized this laboratory-born group. Data from a second study of captive-born monkeys revealed a plausible proximate basis for maintenance of within-matriline cohesion, so characteristic of wild rhesus monkey troops, through changes in the preferred partners of offspring associated with the birth of an infant into the matriline. A third study initiated during the past year has focused on the phenomenon of pubertal male emigration as it occurs within wild-born rhesus monkey troops. Data from this semi-field study will include periodic measures of physical and hormonal maturation and hypothalamic-pituitary-adrenal and psychophysiological response to dominance encounters, in addition to the behavioral measures typically obtained in field studies.           </p>																														

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01108-03 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparative Studies of Play Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. Biben Senior Staff Fellow LCE, NICHD

Other: D. Symmes Head LCE, NICHD  
D. Bernhards Bio. Lab. Tech. LCE, NICHD  
G. Hetzel Animal Caretaker LCE, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Section on Brain, Behavior, and Communication

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.8

## PROFESSIONAL:

1.0

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Social Environment Effects on Play. Data analysis was completed for this study which investigates the immediate effects of manipulating the play behavior of young male squirrel monkeys by restricting the pool of available play companions. The 12 subject males maintained high levels of play activity with four different classes of play partners (including classes normally avoided by them) by adapting their play to each class of partner. This indicates a high degree of social awareness, as well as a strong motivation to play. Our previous studies suggested a major role for normal play interactions in the development of flexibility and resilience in social skills. The present findings suggest that maladaptive personality types like "bullies" and "sissies" may result when play is restricted to inappropriate partners for extended periods. We have concluded that play is of such value to developing monkeys that it will persist even under suboptimal conditions. These studies (which manipulate play without eliminating social contact) have reached a point where definitive studies on the function of play are possible.

B. Vocalizations Used in Play. Our previous study found that the abundant vocalizing of squirrel monkeys during play had very limited communicative significance for the playing animals themselves. We have recently begun to test an alternative function--that such calls signal nearby adults to the presence of play activity. Benefits of such a message include reassurance that the young are engaged in a harmless activity and alerting adults to increase their vigilance for predators while the young are preoccupied in play. Noisy play, which is unusual in animals, may in fact attract the attention of predators and thereby increase the risk involved in play. We are now measuring changes in adult vigilance behavior as a function of play vocal activity.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01110-02 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Intuitive Parenting of Infants in Comparative Perspectives

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: S. J. Suomi Head LCE, NICHD

Other:	H. Papousek	Guest Researcher	LCE, NICHD
	M. Papousek	Guest Researcher	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD
	W. Thompson	Guest Researcher	LCE, NICHD

## COOPERATING UNITS (if any)

Laboratory of Developmental Psychobiology, Max Planck Institute, Munich

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Child and Family Research Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL

0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unraduced type Do not exceed the space provided)

The present project serves two main purposes: (1) to improve knowledge on mental and communicative development in preverbal human infants, focusing attention upon didactic tendencies recently detected in intuitive forms of parental behaviors by Papousek and Papousek; and (2) to characterize similarities and differences related to intuitive parenting in two cultures--Caucasian American and Mandarin Chinese--that differ dramatically in the tonal quality of the adult language forms. Interactions between mothers and their infants at the ages of 2 and 4 months have been videotaped for microanalysis of vocal sound patterns, facial expressions, gestures, and other behaviors involved in mother-infant communication. Spectrographic analysis of vocal sounds has been methodologically enriched (in cooperation with Dr. David Symmes, BBCS, LCE) by introduction of innovative programs facilitating computer-aided analysis of pitch patterns. Data on the total of 15 American and 18 Chinese mother-infant dyads have been collected and from this data set 350 one-minute-samples have been selected macroanalytically and auditively categorized in relation to the main types of interactional contexts. Maternal utterances from these samples have been transcribed, translated by a Chinese linguist, and prepared for microanalytical evaluations in further collaboration with Papousek and Papousek at the Laboratory of Developmental Psychobiology in Munich, Germany. Preliminary findings have revealed striking similarities in patterns of infant-directed utterances by American and Chinese mothers, despite the major differences in multiple dimensions of the two adult language forms. The project has been temporarily inactivated pending the return of the Max Planck collaborators to the LCE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01111-02 LCE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Affecting Nurturant Behavior Toward Infants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.:	F. A. Pedersen	Head	LCE, NICHD
Other:	G. Kestermann	Visiting Fellow	LCE, NICHD
	Y. Bryan	Visiting Fellow	LCE, NICHD
	L. Huffman	NRSA Fellow	LCE, NICHD
	M. Pato	NRSA Fellow	LCE, NICHD
	P. Berman	Guest Researcher	LCE, NICHD
	H. Moss	Guest Researcher	LCE, NICHD
	S. Theut	Guest Researchr	LCE, NICHD

COOPERATING UNITS (if any)

Rockefeller Foundation

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 2

TOTAL MAN-YEARS

5.0

PROFESSIONAL

5.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project encompasses four recently initiated studies dealing with the development of parents' nurturant responses to infants. The first addresses the inter-generational transmission of nurturant roles for females and males. It examines whether parents communicate differential expectations in their play behavior with male and female children regarding care for babies. The hypothesis being tested is that mothers foster stronger nurturant expectations than fathers, while fathers differentiate their role expectations for males and females more strongly than mothers do. The second study involves an intervention during the pregnancy period for first-time expectant mothers. The intervention, which includes having the expectant mother (a) handle a young infant, (b) observe her behavior with the infant on videotape, and (c) receive feedback about her behavior, is hypothesized to reduce anxiety, heighten the mother's sensitivity to different arousal states in the young infant, and facilitate her making appropriate responses to behavior emitted by the infant. The third study compares two groups of expectant parents who differ in exposure to a specific psychological stress, previous pregnancy loss (miscarriage, stillbirth, or neonatal death) in order to determine whether this experience contributes toward anxiety, depression, or dysfunctional parental adaptations. The fourth study is concerned with the mother's emotional state during pregnancy; anxiety and depression during pregnancy are predicted to be related to postpartum affective state, quality of parent-infant interaction, and reactivity to infant cries. Data collection is complete on the first three studies and is in progress on the fourth study.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01112-01 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Development of Individual Differences in Social and Cognitive Competence

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. E. Lamb Head LCE, NICHD

Other: R. D. Ketterlinus Guest Researcher LCE, NICHD

## COOPERATING UNITS (if any)

Center for Human Growth and Development, University of Michigan (F.L. Bookstein)  
Department of Psychology, University of Goteborg, Sweden ( C.-P. Hwang, A. Brogerg)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Section on Social and Emotional Development

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

.14

## PROFESSIONAL:

.14

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

This project is a continuation of a research program initiated by Dr. Lamb at the University of Utah. Work completed or in progress at NICHD involves analyses of data from a longitudinal study in Sweden examining the effects of center day care, family day care, and home care on the development of 140 children recruited at an average of 16 months of age.

A. Social competence. Multivariate analyses using Wold's Partial Least Squares "soft modelling" procedure indicated that type of care had no reliable impact on the children one and two years post-enrollment. The quality of care received at home had the most consistent impact on personality maturity and emergent social skills with peers and adults. The quality of alternative care had a less consistent and more modest effect, as did measures of family social support networks, temperament, and child gender.

B. Intellectual competence. PLS analyses again showed that type of care was unrelated to intellectual competence. Quality of home care was the most important predictor of tested performance one and two years after enrollment; quality of out-of-home care was not predictively important.

The significance of these findings lies in its emphasis on the need to consider not only the type but also the quality of out-of-home care, and to consider the role of factors outside the care setting--such as the quality of home care--when evaluating day care arrangements. This is also the first project to apply the PLS procedure to longitudinal behavioral data.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01113-01 LCE

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: M. E. Lamb Head LCE, NICHD

Other: R. D. Ketterlinus Guest Researcher LCE, NICHD

## COOPERATING UNITS (if any)

Department of Psychology, University of Maryland Baltimore County (D.M. Teti)  
Department of Pediatrics, University of Utah Medical School (A.B. Elster)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Section on Social and Emotional Development

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

.13

## PROFESSIONAL:

.13

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This project is a continuation of a research program initiated by Dr. Lamb at the University of Utah. Work completed or in progress at NICHD involves analyses of data from two large nationally-representative samples: the National Longitudinal Study of Youth (NLSY) and the Census Bureau's Current Population Surveys (CPS). The goal is to describe the psychosocial context of adolescent parenthood and to explore the longterm effects for both mothers and fathers.

A. Correlates of adolescent fatherhood. In an analysis of data from the NLSY, we found clear evidence that, regardless of race, adolescent fatherhood was but one symptom of a wider variety of psychosocial problems. Compared with nonfathers of similar ages from similar backgrounds, adolescent fathers were much more likely to have a history of involvement with police, school problems, and substance abuse.

B. Long-term correlates of adolescent parenthood. In a previous analysis of CPS data, it was shown that adolescent marriage was associated in men with deficits in marital stability, income, educational attainment, and occupational prestige through through at least 40 years after the marriage. Similar adverse long-term consequences were subsequently found for mothers. In the case of mothers, both adolescent childbearing and adolescent marriage were associated with higher lifetime fertility (adjusting for age), lower income, less prestigious occupational ratings, lower educational attainment, and more frequent marital dissolution. The effects were not additive, but the "best" outcomes were obtained by those women who delayed both childbearing and marriage into adulthood.

The significance of this project lies in its demonstration that adolescent parenthood (a) is not a random or chance event and (b) has long term--essentially life-long--effects on the psychological and socioeconomic status of both men and women.



LABORATORY OF DEVELOPMENTAL AND MOLECULAR IMMUNITY

- Z01 HD 00073-16 Regulation of Immune Systems at the Cellular Level  
Edgar E. Hanna, Ph.D.
- Z01 HD 00920-06 Molecular Structure of Mouse Histocompatibility  
(H-2) Genes:  
Keiko Ozato, Ph.D.
- Z01 HD 01301-05 Human Immune Response to Polysaccharide-Protein  
Conjugate Vaccines  
Rachel Schneerson, M.D.
- Z01 HD 01304-05 Protective Effect of Vi Polysaccharide Antibodies Against  
Typhoid Fever  
John B. Robbins, M.D.
- Z01 HD 01306-04 Pertussis Heat Labile Toxin (HLT): Isolation and  
Characterization  
Ronald D. Sekura, Ph.D.
- Z01 HD 01307-04 Pertussis Toxin: An Approach to a New Pertussis Vaccine  
Ronald D. Sekura, Ph.D.
- Z01 HD 01308-04 Conjugation of Pneumococcal Vi Polysaccharides with  
Carrier Proteins  
Shousun C. Szu, Ph.D.
- Z01 HD 01310-01 Developmental Gene Regulation of the Immune System  
Keiko Oazto, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Laboratory of Developmental and Molecular Immunity

Research is conducted into the developmental molecular biology as cellular basis of immunity especially in the fetus infant and child age groups.

The Section on Bacterial Disease Pathogenesis and Immunity has been primarily concerned with vaccine development for the prevention of encapsulated bacterial diseases especially Haemophilus influenzae type B and typhoid fever and for the toxin-mediated disease pertussis. The organisms that cause these diseases are not habitants of nor pathogens for humans; verification therefore, of the protective nature of antigens of these pathogens as well as a host of immune factors that confer resistance to disease requires clinical trials with vaccines. In most cases, investigational vaccines for the study of these problems must be prepared by the LMDI in a clinically satisfactory manner.

The development of a synthetic scheme to prepare capsular polysaccharide-protein conjugates for inducing antibodies in infants as well as immunologically deficient patients was first described by Schneerson, Robbins, et al. Their synthetic scheme, as well as other methods for preparing polysaccharide protein conjugates, are now under study in many laboratories. The Haemophilus influenzae type b - tetanus toxoid conjugate synthesized by LMDI has now proven to be at least 5 to 10 times more immunogenic than the Haemophilus influenzae type b capsule of polysaccharide alone. A study in Swedish children, using the U.S. licensed Haemophilus influenzae type b capsule of polysaccharide vaccine in a side-by-side comparison of the Haemophilus influenzae type b tetanus toxoid conjugate, showed the latter to be 11 times more immunogenic as the polysaccharide alone, as measured by geometric mean post-immunization levels of antibodies, and to induce protective levels of antibodies in all the recipients. The Haemophilus influenzae type b capsule of polysaccharide alone, in contrast, induced protective levels of antibodies in only 70% of the children. Efficacy trials of this investigational conjugate vaccine are now underway in infants and children in Charlotte, N.C. and Goteborg, Sweden.

The problem of developing a new typhoid vaccine in the United States has been significant; Salmonella typhi, the cause of two organisms, is an inhabitant of and a pathogen for humans only. Therefore, an improved vaccine for typhoid requires clinical investigation. Since typhoid is not found in the United States, the effectiveness of candidate vaccine for typhoid must be evaluated in a clinical setting in which the attack rate is sufficiently high and surveillance is reliable enough to evaluate the protective effect of an investigational product. In a double-blinded, randomized clinical trial of the Vi capsular polysaccharide, using pneumococcal vaccine as a control polysaccharide vaccine, an effectiveness trial was conducted in about 7,000 subjects in Kathmandu Valley, Nepal, age 5 to 44 years of age. After 17 months of surveillance, the effectiveness of the Vi vaccine was confirmed. The p value of 0.0004 for the attack rate of typhoid fever in the Vi injected group versus that of the pneumococcal injected group confirms the hypothesis set forth several years before that Vi antibodies would confers protection against typhoid fever. This study has

prompted two additional research programs; 1) to improve the efficacy of the Vi, and to make it suitable for infants and children, by covalently binding it to a protein in a clinically acceptable manner; 2) to prepare the carbohydrate antigens of other causes of enteric fevers using the same technology.

The hypothesis that pertussis is a toxin mediated disease provided the intellectual background for the development of a vaccine composed of a single protein, pertussis toxin inactivated to form pertussis toxoid. Pertussis toxin is oligomeric protein composed of an alpha subunit (enzymatically active) and beta (binding subunits). Two problems were encountered in developing this investigational vaccine; 1) the cultivation of Bordatella pertussis and the purification of the toxin by traditional means provided low yields of materials unsuitable for clinical investigation; 2) the alpha subunit of pertussis toxin does not contain lysine, the principle amino acid reactive with formaldehyde (well established method for inactivating other bacterial toxins such as diphtheria and tetanus toxins). Techniques were modified to cultivate Bordatella pertussis by new methods in large scale fermentation and to purify the pertussis toxin by affinity chromatography. The resultant product was inactivated by a novel method for biologics; treatment under controlled conditions with hydrogen peroxide. Treatment with hydrogen peroxide exerts several effects upon the amino acid composition of proteins that are irreversible in vivo and in vitro. The newly developed pertussis toxoid was shown to have no residual toxin activity, by both in vivo and in vitro assays, and to induce antibodies and protection in laboratory animals. A clinical investigation of this pertussis toxoid in adults and 18 month old children, no local reactions or fever were observed. A dose-related immune response was observed; 1) 50 mg yielded a maximum response as measured by postimmunization geometric mean levels and protective levels of antibody, as measured by neutralization assays, in all the recipients, 2) reinjection of the toxoid did not induce a booster response. The levels of antibodies in the adults were equal to or higher than adults convalescent from pertussis and in 18 month old children following their fourth injection of DTP vaccine. The conclusions from these studies so far is that the NICHD pertussis toxoid is safe and immunogenic. Its immunogenicity is at least as equal to that of disease and greater than that induced by the current whole cell vaccines. Plans continue for evaluating the effectiveness of the pertussis toxoid in Sweden and in Massachusetts.

Research in the Section on Molecular Genetics of Immunity is directed toward understanding both the development, the structure and regulation of class I major histocompatibility (MHC) antigens on a molecular level in the murine system. Dr. Ozato has accumulated a library of monoclonal antibodies whose epitope specificity for the class I MHC antigens has been characterized. She has also increased the scope of their studies by refinement of the site-directed mutagenesis.

The unique and diverse genetic polymorphism of class I MHC antigens, their essential role in immune responsiveness and resistance against both malignancy and infections are features which make the study of this structure critical to our understanding of the development of immunity. Ozato and her colleagues continue to study the structure/function relation and regulation of class I MHC gene expression. Structure/function relationships of MHC are primarily studied by site-directed mutagenesis. Targeted mutations are introduced by a new efficient method in which



unmutated template sequences are eliminated before screening for the new MHC genes are transfected. Mutants are sequenced by the dideoxy method after they have been introduced and expressed into mouse L-fibroblasts. The antigenic activities, against mouse lymphoid cells with cytotoxic specificities and monoclonal antibodies are then examined. The domains of the MHC antigen responsible for these critical immune functions have been identified. More than half of the relevant H-2D<sup>d</sup> S-epitopes (recognized by monoclonal antibodies) have been identified in a small stretch of amino acids in this position. C-determinants, recognized by cytotoxic T-lymphocytes find some overlap but unique positions on the MHC antigens. More detailed mapping of these allo-epitopes of MHC class I antigens by intradomain recombinants were developed. The specificity of monoclonal antibodies were examined. L-cells transfected with recombinants between H-2K<sup>d</sup> and H-2D<sup>d</sup>. These recombinants have provided, demonstration that allo-antigens are formed on MHC antigens by portions of different domains. The fact that different domains can contribute to the formation of a single epitope now provides a insight for understanding the three dimensional structure of the MHC class I antigens.

The regulation of expression of the class I MHC genes were studied by two methods. The first, assays in details a 310 base paired DNA fragment corresponding to the 5' flanking region of the class I MHC genes. In this region, two regulatory elements have been found: 1) The class I regulatory element and the interferon consensus sequence. Both of these regulatory elements increased the expression of class I MHC antigens. Of particular interest was the observation that the c-fos regulation increased right before, during and immediately after birth in the mouse. Unexpectedly, with the increase in c-fos activity was also an increase in another oncogene or regulating element, c-myc. The relation between these two regulatory elements is under study. Another discovery by Ozato and her colleagues was that there appeared DNA binding proteins with important effects upon the expression of the class I MHC antigens. The structure of these binding proteins is being studied in order to provide an experimental approach to locate their genetic origin and thereby, to study their control of MHC class I antigens.

The Section on Immunoregulation and Cellular Control cloned T-cell hybridomas have been used to study the regulation of immune responses. Precursor T-cell hybridomas have been exposed to several bacterial toxins, including pertussis toxin, in order to understand the cellular, and ultimately molecular basis, for the actions of these immunoregulatory molecules. Clinical to the pathogenesis of pertussis, is the high incidence of serious respiratory infections that occur during the disease. The immunoregulatory role of pertussis toxin has been shown but the details which underlay its sharp dose response relation and the cell type involved remain unclear. Hanna has showed that pertussis toxin treated T-cell hybridomas have a shift in their predicted phenotype. These studies will be extended, in order to understand the effect of this altered phenotype upon more differentiated cells and ultimately in vivo. A toxin of a gram-positive pathogen, the streptococcal pyogenic toxin, will also be investigated with this method. Both toxins are not cytotoxic but have pharmacologic actions, including those expressed upon these T-cell hybridomas, which facilitate their in vivo and in vitro studies.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 00073-16 LDMI
<b>PERIOD COVERED</b> October 1, 1986 to September 30, 1987		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Regulation of Immune Systems at the Cellular Level		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>		
PI:	Edgar Hanna	Head LDMI, NICHD
Others:	Prince Arora	Staff Fellow LDMI, NICHD
	Michael Walker	Biologist LDMI, NICHD
	Ronald Sekura	Research Chemist LDMI, NICHD
<b>COOPERATING UNITS (if any)</b> P. Skolnick, LN, NIDDK; C. Hansen, VR, DRS		
<b>LAB/BRANCH</b> Laboratory of Developmental and Molecular Immunity		
<b>SECTION</b> Section on Immunoregulation and Cellular Control		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland, 20892		
TOTAL MAN-YEARS 2.75	PROFESSIONAL: 1.75	OTHER: 1.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)</b> <p>           Precursor and mature regulatory T-lymphocyte hybridomas have been constructed and cloned from splenocytes of nude, +/nu, and +/+, NFR/N mice. Mature effector (CTL) clones were raised from +/+ splenocytes in the presence of antigen and growth factors. These clones are exploited as experimental cellular models to facilitate delineation of pathways for understanding activation, modulation or deregulation of immune systems by microorganisms. <u>B. pertussis</u> toxin (PT), <u>S. pyogenes</u> exotoxin (SPE), and <u>S. typhimurium</u> endotoxin (ET) were used as probes in a cell complementation model <u>in vitro</u> involving B-lymphocytes, from nude mice as responders, and the appropriate regulatory cells in question (fractionated T-cells, or their cloned representatives). PT and SPE were suppressive for CTL responses to alloantigens present during the initial 1-2 hr of CTL generation. Suppression was reversible during this period but not later. Suppression was dose dependent and was optimally generated over a dose range of 0.05-1.0 <math>\mu\text{g}</math> PT/<math>10^6</math> cells. SPE, a known PFC suppressant, required 10- to 100-fold greater concentrations to suppress CTL. Neither toxin was directly cytotoxic. PT, but not SPE was mitogenic for the fractionated <math>\text{Ig}^-</math>, T-cells at lower concentration. Neither toxin was active on mature CTL nor on mature regulatory clones. PT generated and increased the number of <math>\text{Thy1}^+</math>, <math>\text{L3T4}^-</math>, <math>\text{Lyt1}^-</math>, <math>\text{Lyt2}^+</math> suppressor cells for CTL from splenocyte precursors. SPE deactivated and selected against the suppressive activity of a <math>\text{Thy1}^+</math>, <math>\text{L3T4}^+</math>, <math>\text{Lyt1}^+</math>, <math>\text{Lyt2}^+</math> T-cell precursor clone. ET effected neither CTL generation, nor the T-cell clones at conventional concentrations. This data indicate that PT generates differentiation of suppressor T-cells for CTL. SPE reverses the differentiation of suppressor T-cells for PFC.         </p> <p>           Tumorigenic hybrid clones have also been exploited as models to investigate the <u>in vivo</u> efficacy of CTL in semi-syngeneic nude hosts. Adoptive CTL conferred weak short-long term (cell number dependent) protection which was assisted by partially defined antibodies arising in the nude mice undergoing long-term (&gt;10 mth) resistance.         </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00920-06 LDMI

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Structure of Mouse Histocompatibility (H-2) Genes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Keiko Ozato Head LDMI, NICHD

Others: Peter Burke Intramural NRSA LDMI, NICHD  
Yasuaki Shirayoshi Visiting Fellow LDMI, NICHD

## COOPERATING UNITS (if any)

E. Appella, LCB, NCI; J. Forman, Department of Microbiology, University of Texas, Dallas, Texas

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Molecular Genetics of Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.5

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Major histocompatibility complex (MHC) class I genes encode highly polymorphic transplantation antigens that are essential for immune recognition of foreign antigens. MHC antigens also invoke rejection of transplanted tissues. Contrary to most genes, the coding regions of MHC class I genes contain more nucleotide substitutions than introns, which signifies evolutionary selection of this high degree of polymorphism. The polymorphism seen at the level of nucleotides is the basis of numerous antigenic epitopes that are expressed on the MHC class I antigens, which plays an essential role in their immune functions. We studied epitope organization of MHC class I genes by site-directed mutagenesis. Based on our postulate that amino acids from position 63 to 73 of MHC antigens are important for formation of epitopes, mutations are introduced into the H-2L<sup>d</sup> gene to replace codons of this region with those of the H-2D<sup>d</sup> antigen. Examination of the phenotypes of the mutated genes expressed in fibroblasts confirmed that this region indeed forms a major site for epitopes. The power of this general approach in which predictions for a functional site of a molecule can be reliably tested is verified. We have extended site directed mutagenesis to delineate structure-function relationships of the highly conserved regulatory element (CRE) and interferon consensus sequence (ICS) of MHC class I genes. These structures are found in sequences upstream from the start site of RNA synthesis. The CRE is involved in developmental regulation of the MHC gene expression, and the ICS plays a role in the increased expression of the genes after interferon treatment. Site directed mutagenesis is introduced systematically along the entire CRE and ICS, and then connected to a reporter gene CAT. Functional activity of mutated sequences is assessed in a variety of cells by measuring CAT activity. The ability of the sequences to interact with nuclear factors is also evaluated by gel mobility shift analysis. These efforts provide us with insight into how the regulatory sequences govern regulated expression of the MHC class I genes.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01301--05 LDMI
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Human Immune Response to Polysaccharide-Protein Conjugate Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Rachel Schneerson	Senior Investigator LDMI, NICHD
Others:	John B. Robbins	Head LDMI, NICHD
	Yong Hong Yang	Visiting Fellow LDMI, NICHD
COOPERATING UNITS (if any) G. Schiffman, State University, NY; J.C. Parke, Jr., Charlotte Memorial Hospital, NC; J. Schlesselman, USUHS, Bethesda, MD; B. Trollfors, J. Taranger, B. Claesson, T. Lagergard, University of Goteborg, Sweden; C. Lowe, OD, NICHD; D. Bryla, EBRP, NICHD.		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity		
SECTION Section on Bacterial Disease Pathogenesis and Immunity		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland, 20892		
TOTAL MAN-YEARS 3.9	PROFESSIONAL: 3.9	OTHER 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The age-related and T-independent properties of <u>Haemophilus influenzae</u> type b capsular polysaccharide (Hib CPS) limit its protective actions to children older than 24 months; its effectiveness in children ages 18 - 24 months has not been established. Hib CPS is neither immunogenic or protective in children less than 18 months old, that age group with the highest attack rate of Hib meningitis. A clinically acceptable scheme was devised to bind Hib CPS and other CPS antigens to proteins. Conjugates of Hib CPS polysaccharide (CPS) bound to tetanus toxoid (TT) by this method were considerably more immunogenic than CPS alone in rodents, in juvenile and infant rhesus, and in adult volunteers. The isotypes and biological activities of antibodies elicited by these conjugates were similar to those induced by CPS vaccines. The safety and immunogenicity of Hib CPS alone, or Hib-TT conjugates, either fluid or adsorbed, were evaluated in 18-23 months old healthy children in Goteborg, Sweden. The lowest rate of side reactions was elicited by Hib CPS; adverse reactions elicited by the two conjugates was similar. Hib-TT fluid was the most immunogenic of the 3 vaccines eliciting about 9 times the levels of antibodies than Hib CPS alone. Hib-TT elicited protective levels of Hib CPS and ATT antibodies in 28/28 18-23 months old children with one injection; these antibodies exerted biological properties that have been correlated with immunity. The greatest fold increase was in IgG followed by IgM and IgA. Rises in IgG1 subclass of Hib CPS antibodies were the most frequent followed by IgG2; some children had rises in IgG3 and IgG4. Vaccine-induced Hib CPS antibodies were bactericidal. Hib-TT fluid also elicited higher levels of anti-TT than Hib-TT adsorbed; these anti-TT neutralized tetanus toxin in-vivo. Clinical evaluation of these conjugates is planned for immunodeficient patients and in 2 months old infants, the target population for prevention of Hib meningitis.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01304-05 LDMI

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John B. Robbins	Head	LDMI, NICHD
Others:	Shousun Szu	Senior Staff Fellow	LDMI, NICHD
	Rachel Schneerson	Senior Investigator	LDMI, NICHD
	Tod Cramton	Chemist	LDMI, NICHD

COOPERATING UNITS (if any) H. Kornhof, African Institute of Research; I.L. Acharya, Infectious Disease Hospital, Kathmandu, Nepal; R. Kumar, All India Institute of Medical Sciences; C. Lowe, OD, NICHD; D. Bryla, EBRP, NICHD; M. Cadoz, Institut Merieux, Lyon, France.

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Bacterial Disease Pathogenesis and Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

0.3

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Enteric fevers, of which typhoid fever is the most common, remain a serious and frequent cause of morbidity and mortality in most underdeveloped nations. The protective role of antigens of the salmonellae are difficult to evaluate because Salmonella typhi, the causative agent of typhoid fever, is a pathogen and inhabitant of humans only. Two, double masked, randomized, controlled evaluations of the capsular polysaccharide of S. typhi (Vi) as a vaccine to prevent typhoid fever is in progress in Nepal and in the Eastern Transvaal, RSA. No significant side reactions were elicited by the Vi in pilot study of 274 Nepali; about 75% responded with a  $\geq$  4-fold rise in serum antibodies. Residents of 5 villages were injected IM with either Vi or pneumococcus vaccine (control). There were 6907 participants of which 6,438 were in the target population (ages 5 - 44 years); each was visited every 2 days. Those with fever of 100°F or higher for 3 consecutive days were asked to give blood for culture. Typhoid was diagnosed as blood culture-positive or clinically-suspect, based upon bradycardia, splenomegaly, and fever. The annual attack rate of typhoid was 12.2/1000 in the controls and 3.2/1000 in the Vi-immunized group ( $p < 0.0001$ ). The efficacy of the Vi was 69% for culture-positive cases, 77% for suspect cases and 73% for these 2 groups combined. In collaboration with Drs. Keith Klugman and Hendrik Koornhof, 11,400 school-age children, most 5 to 12 years of age, received either Vi or bivalent meningococcal vaccine. In the 16 months after immunization, 34 cases of typhoid fever were detected in the controls and 13 cases in the Vi group ( $p = 0.0004$ , vaccine efficacy 71%). These data provide evidence that Vi antibodies confer protection against typhoid. Surveillance continues to determine the duration of Vi-induced immunity. The LPS of S. paratyphi A, has been purified and detoxified; its immunogenicity and potential for preventing the next most common cause of enteric fevers is under evaluation.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 01306-04 LDMI
<b>PERIOD COVERED</b> October 1, 1986 to September 30, 1987		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Pertussis Heat Labile Toxin (HLT): Isolation and Characterization		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>		
PI:	Ronald Sekura  Yan-ling Zhang Robin Roberson Xiuru Li	Research Chemist  Visiting Associate Chemist Visiting Fellow  LDMI, NICHD  LDMI, NICHD LDMI, NICHD LDMI, NICHD
<b>COOPERATING UNITS (if any)</b>  None		
<b>LAB/BRANCH</b> Laboratory of Developmental and Molecular Immunity		
<b>SECTION</b> Section on Bacterial Disease Pathogenesis and Immunity		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland, 20892		
<b>TOTAL MAN-YEARS:</b> 2.0	<b>PROFESSIONAL:</b> 2.0	<b>OTHER:</b> 0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)</b>  <p> <u>Bordetella pertussis</u> produces several proteins, in addition to pertussis toxin, with pharmacological activities (toxins). One of these, previously called the dermonecrotic toxin and now designated as heat labile toxin (HLT), has been isolated in highly purified form. Its structural and biological activities are being characterized. HLT is composed of a single polypeptide chain, molecular weight ca. 150,000 D. The lethal dose in laboratory mice is unusually low, ranging about 10 pg, making it one of the most toxic proteins studied. Monoclonal antibodies have been isolated which confer immunity to intracerebral challenge of mice with <u>B. pertussis</u> providing evidence that HLT might serve as an additional protective antigen.         </p> <p>           Injection of sublethal doses of HLT induces unusual pathological changes including rapid depletion of the red marrow. <u>B. pertussis</u> produces only trace amounts of HLT, accordingly, DNA recombinant technology must be applied to this problem of production in order to consider the use of this toxin for clinical evaluation.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01307-04 LDMI

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Pertussis Toxin: An Approach to a New Pertussis Vaccine

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald Sekura	Research Chemist	LDMI, NICHD
Others:	Yan-ling Zhang	Visiting Associate	LDMI, NICHD
	Nathaniel Tolson	Biologist	LDMI, NICHD
	Robin Roberson	Chemist	LDMI, NICHD

## COOPERATING UNITS (if any)

J. Shiloach, B. Kaufman, NIDDK; B. Trollfors, Univ. of Goteborg, Sweden; G. Siber, Massachusetts Public Health Laboratories, Jamaica Plains, MA.

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Bacterial Disease Pathogenesis and Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

## TOTAL MAN-YEARS:

2.67

## PROFESSIONAL:

1.07

## OTHER:

1.6

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The incidence and severity of pertussis have been controlled by widespread immunization with DTP which contains inactivated Bordetella pertussis organisms (cellular vaccine). The identification of pertussis toxin, an extracellular protein of this pathogen, as a major, if not the sole protective antigen, afforded an opportunity to produce a new vaccine with improved safety and efficacy. B. pertussis was cultivated in a 100L fermenter and the pertussis toxin extracted from the culture supernatant by affinity chromatography. The pertussis toxin was converted to a toxoid by controlled inactivation with hydrogen peroxide. The resultant toxoid, NICHD-PTxD, was shown to have less than 1% of its original binding and enzymatic activity in in-vitro assays. In-vivo assays, which require both binding and enzymatic activity on the same molecule, showed no detectable activity. A lot of NICHD-PTxD, PTH-06, was adsorbed onto aluminium salts and three doses, 10, 50 and 75 micrograms, was evaluated in adult volunteers at the Clinical center of the NIH and then in 18 months old children, previously injected with DTP during infancy, at the Children's Hospital Medical Center, Boston, MASS and in Goteborg, SWEDEN. The adults were injected twice, six months apart with either of the 3 doses; none had fever, significant local reactions, lymphocytosis, or altered insulin or glucose levels (attributable to the vaccine). Serum antibodies induced by the vaccine neutralized pertussis toxin in-vitro and the levels achieved by the 50 microgram dose (maximum response) were higher than those detected in adults convalescent from pertussis. No significant adverse reactions or fever were observed in the 18 months old; the levels of pertussis toxin antibodies were significantly higher than those in age-matched controls injected with DTP. NICHD-PTxD has been shown to be compatible with DT and a formulation of this new infant vaccine is under investigation.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01308-04 LDMI
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Conjugation of Pneumococcal and Vi Polysaccharides with Carrier Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Shousun Szu	Senior Staff Fellow LDMI, NICHD
Others:	John B. Robbins	Head LDMI, NICHD
	Ali Fattom	Visiting Fellow LDMI, NICHD
COOPERATING UNITS (if any) J.L. Inman, LI, NIAID; W. Vann, OBRR, FDA; W. Karakawa, Department of Biochemistry, Pennsylvania State University, PA.		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity		
SECTION Section on Bacterial Disease Pathogenesis and Immunity		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland, 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.3	2.3	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The Vi capsular polysaccharide of <u>Salmonella typhi</u> (Vi), has proven to be a protective antigen in two double masked, controlled clinical trials in areas with high rates of typhoid fever. The efficacy of the Vi was about 70% in both studies. The Vi elicited a 4-fold or greater rise of serum Vi antibodies in 98% of U.S. and French volunteers. Only 75% of vaccinates in these areas with high typhoid fever, and who had other chronic infectious diseases and varying degrees of malnutrition, responded with a 4-fold or greater rise. Methods were devised, therefore, to synthesize Vi-protein conjugates in order to both enhance the antibody response and confer T-dependent properties to the Vi (and theoretically increase its protectiveness in populations at high risk for typhoid fever). The heterobifunctional cross-linking reagent, N-succinimidyl 3-(-2-pyridyldithio) propionate (SPDP), was used to bind thiol derivatives of the Vi, and other polysaccharides with carboxyl functions, to proteins. This synthetic scheme was reproducible, provided high yields of V conjugates, and was applied to several medically relevant proteins such as diphtheria and tetanus toxoids, and cholera toxin and its non-toxic beta subunit. The Vi conjugates were more immunogenic in mice and juvenile Rhesus than the Vi alone. Conjugates of Vi induced booster responses in mice and in juvenile rhesus monkeys. Clinical studies with Vi-protein conjugates are planned. This scheme was applied to pneumococcus type 12F, which contains an aminouronic acid, as a model for preparing conjugates of <u>Staphylococcus aureus</u> capsular polysaccharides. method of activation, using SPDP, was successfully adapted to this model polymer and the resultant type 12 conjugates were more immunogenic and induced booster responses compared to the polysaccharide alone. The difficult-to-prepare <u>S. aureus</u> capsules are now under study.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01310-01 LDMI

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Gene Regulation of the Immune System

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Keiko Ozato	Head	LDMI, NICHD
Others:	Ben-Zion Levi	Visiting Associate	LDMI, NICHD
	Kazushige Hamada	Visiting Fellow	LDMI, NICHD
	Paul Driggers	IRTA	LDMI, NICHD
	Jun-ichi Miyazaki	Visiting Associate	LDMI, NICHD
	Bonnie Orrison	Chemist	LDMI, NICHD
	Toby Silverman	NRSA	LDMI, NICHD
	John Kasik	NRSA	LDMI, NICHD

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Molecular Genetics of Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

## TOTAL MAN-YEARS

5.3

## PROFESSIONAL:

4.3

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The establishment of the fully functional immune system requires precisely programmed regulation of gene expression that occurs at many different levels. As a model for the regulation of multiple genes in development we study expression of an oncogene *c-fos*. This oncogene encodes a DNA binding nuclear protein implicated to have a role in controlling expression of other genes. We also work on transcriptional control of major histocompatibility complex (MHC) class I gene expression to elucidate detailed mechanisms of developmental regulation. We have found that expression of *c-fos* oncogene in the mouse is induced transiently at the day of birth. This induction is observed in all the neonatal organs tested. We also found that *c-fos* oncogene is induced in a T-cell hybridoma upon antigenic stimulation and by interferon treatment. These and other results are consistent with the proposed regulatory role of *c-fos* gene in development, and prompted us to explore a means of manipulating the *c-fos* gene expression *in vitro* by employing anti-sense constructs. The *c-fos* coding sequence in the reverse orientation was placed under a strong RSV promoter, and introduced into embryonal carcinoma cells. We found that expression of the anti-sense RNA results in almost complete blockade of the endogenous *c-fos* gene expression induced by interferons and by other stimuli. This study demonstrates the validity of using anti-sense constructs to study function of a gene, since it allows to control gene expression *in vitro*. For MHC class I gene expression, we have shown the presence of trans-acting nuclear factors that interact with the regulatory region (CRE) of a MHC class I gene. The CRE is highly conserved among and controls developmental expression of MHC class I genes. By using gel mobility shift analysis we found that there are three distinct binding sites in DNA to which independent factors bind. The nuclear factors appear to correlate with high level expression of the MHC gene. Having analyzed detailed binding site by methylation interference tests, we are in the process of isolating and studying the binding proteins.





## LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

- Z01 HD 00047-18 Biochemical Studies of Neuronal and Other Cell Types  
Douglas E. Brenneman, Ph.D.
- Z01 HD 00048-13 Transcriptional-level Control of Neurobiologic  
and Development Phenomena  
Bruce K. Schrier, M.D., Ph.D.
- Z01 HD 00064-11 Neurobiologic Studies of Neurons and Glia in Cell Culture  
Phillip G. Nelson, M.D., Ph.D.
- Z01 HD 00094-17 Pineal Regulation: Environmental and Physiological  
Factors  
David C. Klein, Ph.D.
- Z01 HD 00095-17 Pineal Regulation: Transsynaptic and Intracellular  
Mechanisms  
David C. Klein, Ph.D.
- Z01 HD 00704-03 Tetanus Toxin Effects and Localization in Neurons  
(Inactive)
- Z01 HD 00706-02 Physiological Studies of Nervous System Development  
In Vitro  
(Inactive)
- Z01 HD 00707-03 Pharmacological Studies of Synaptic Transmission  
In Vitro  
Mark L. Mayer, Ph.D.
- Z01 HD 00708-03 Morphologic Studies of Neuronal and Non-Neuronal Cells  
in CNS Cell Cultures  
Elaine A. Neale, Ph.D.
- Z01 HD 00709-01 Prevention of Neuronal Deficits Associated with AIDS  
Douglas E. Brenneman, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Laboratory of Developmental Neurobiology

The Laboratory of Developmental Neurobiology is currently composed of the following Sections and Units:

- 1) Section on Neurobiology headed by Dr. Phillip Nelson is concerned with cellular and molecular mechanisms important for nervous system development.
- 2) Section on Neuroendocrinology headed by Dr. David Klein. Focuses on the pharmacology and molecular and cell biology of the pineal gland.
- 3) Unit on Molecular Neurobiology headed by Dr. Bruce Schrier studies gene expression during the development of neural tissue.
- 4) The Unit on Cell Biology headed by Dr. Elaine Neale uses morphological and cell biologic methodologies in analyzing neurodevelopment.

Work in the Section on Neurobiology continues on membrane mechanisms related to synaptic transmission in cell culture model systems of the mammalian central nervous system. We have emphasized postsynaptic receptors for excitatory amino acids and presynaptic mechanisms related to the regulation of neurotransmitter release. The role of peptide and excitatory amino acids in mediating activity-dependent neuronal survival and development is under investigation. Glial cells are involved in this process, releasing neurotrophic materials when activated by neuropeptides and probably other ligands. The cell biology of glial activation and molecular biological and biochemical approaches to isolation and identification of glia-derived neurotrophic factors are active areas of research. The functioning of this portion of the LDN has been strengthened by the appointment of Dr. Elaine Neale as Head of the Unit on Cell Biology. Dr. Brenneman's appointment as a permanent member of the LDN has been approved and he will be heading a Unit on Neurochemistry. We are planning to secure a Visiting Scientist appointment with intent for tenure for Dr. Mark Mayer to head a Unit on Neurophysiology and Biophysics. In conjunction with the Unit on Molecular Neurobiology, these groups will constitute a comprehensive multidisciplinary laboratory involved in analyzing central nervous system development and function.

Dr. David Klein's Section on Neuroendocrinology continues its pioneering studies of pineal function as a model of neural regulation of cell function. His group has focused increasingly on the cell biology of the transduction involved in the catecholaminergic stimulation of the pinealocyte. The dynamic interaction of  $\alpha_1$  and  $\beta$ -adrenoceptors has become amenable to

detailed analysis, and the mechanisms involved in the synergic 'switch-like' function of these two receptor systems described. Regulation of adenylyl and guanylyl cyclases play a central role in this scheme; a number of cytoplasmic and membrane components including protein kinase, phospholipases and intracellular calcium are involved in this regulation. These cell biologic mechanisms are the means of coupling neural activities to regulated gene expression and the pineal system is an extremely favorable model for gaining understanding of this important process.

The Molecular Neurobiology Unit has made progress on isolating differentiation specific genes from neuroblastoma cells. More intense collaboration with the neurochemistry group has been initiated with the goal of isolating neurotrophic materials from glial cells and obtaining the genes for these agents.

#### Neuron-glia-neuron peptide mediated interactions

The Neurochemistry Unit of the LDN has focused on two broad aspects of neurodevelopment: neuronal survival and the regulation of choline acetyltransferase. These studies have emphasized the importance of support cells in the CNS, specifically astroglia, as they relate to these regulatory processes. During the course of these studies and from studies in other laboratories, it has also become apparent that astroglia have a variety of receptors that are coupled into the metabolic and cell biologic activities of the glial cell. We have now shown that communication between neurons and glia occurs in part through the action of neuropeptides. Vasoactive intestinal peptide (VIP), after being released from neurons, stimulates receptors on astroglia, which then make available more trophic material for developing neurons. The mechanism through which VIP produces these effects on astroglia have been investigated by studies of ligand binding to VIP receptors, calcium flux studies, phosphoprotein analysis and measurement of glial mitosis. We have shown that peptidergic stimulation of glia results in increased metabolic activity and protein secretion, as well as increased mitotic activity specifically in the glial population. It seems evident that the variety of receptors on glial cells and the second messenger activation by different agonists provide mechanisms by which glial activation by several neuronal systems could be mediated. Significant progress has been made on the isolation of the glia-derived neurotrophic factors and adequate assays for these factors have been developed. We view this neuropeptide model of neuron-glia-neuron interaction as of fundamental importance in our search for the molecular basis of neuronal survival. Drs. Alderson and Butler have obtained evidence that when the total RNA from nonneuronal cells is translated by frog oocytes, the resulting protein produces a 3-fold increase in choline acetyltransferase activity and up to a 4-fold increase in neuronal surface membrane. Both molecular biological and biochemical approaches are being used to isolate

the glial-derived trophic materials. The cholinergic neurons of the septal region have proven to be a favorable experimental system and comparison between the responses of septal and spinal cord neurons will be useful.

Previous work by Dr. Brenneman's group has shown that the survival of neurons is influenced by not only the trophic factors described above, but also electrical activity. Current work has addressed the role of excitatory amino acids in regulating neuronal survival in developing cultures. Blockade of NMDA receptors by the specific antagonist, AP-5, was found to decrease neuronal survival at micromolar concentrations, while at lower concentrations survival was enhanced. Physiological studies done in conjunction with this work have suggested that perhaps the pattern of electrical activity is of more importance in regulating survival rather than activity per se. The low dosage survival promoting factor of AP-5 is an important topic for further research.

#### Neural effects of coat protein and related peptides

Dr. Brenneman's group has shown that the envelope protein (gp120) of the human immunodeficiency virus (HIV) can produce neuron death in dissociated cultures from the spinal cord and hippocampus. This work was initiated because of a remarkable sequence homology which is present in VIP and gp120 coupled with the demonstrated importance of VIP to neuronal survival. The death associated with gp120 treatment could be prevented by the addition of exogenous VIP or Peptide T, a drug which is currently undergoing tests in humans as a treatment for AIDS. In addition, monoclonal antibodies to the L3T4 receptor have been shown to be effective in preventing gp120-induced neuronal death. Thus, these studies indicate that dissociated cultures may provide a valuable experimental model to study the mechanisms involved in the progressive dementia and neuronal loss associated with AIDS infection.

#### Synapse formation and diminution

Progress has been made in defining a new experimental system involving a multicompartamental culture system. Reproducible innervation of spinal cord (SC) neurons in the center chamber by axons of dorsal root ganglion (DRG) neurons growing in from the side chambers has been achieved. Reliable retrograde labelling of the DRG neurons that so project has been done with fluorescent latex microspheres. Chronic (4-7 days) stimulation experiments have begun and preliminary results indicate that the relative efficacy of stimulated axons is increased by such activation. A well qualified Post-doctoral Fellow, Dr. Douglas Fields, has joined Dr. Neale's Unit and further anatomical and physiological studies are in progress. This is an extremely active and important area in developmental neurobiology and the mechanistic studies we wish to pursue are badly needed.



### Voltage-sensitive calcium channels (VSOC) and transmitter release

VSOC are heterogeneous, and different tissues express VSOC with different properties. Within a given neuron, more than one type may be expressed and these may occur at different regions of the cell surface and presumably subserve different functions. Our previous studies had shown that BayK 8644 acts as a calcium agonist regarding calcium currents evoked under voltage clamp in the neuronal cell body of cultured neurons, but did not augment transmitter release from the axonal endings of these cells. The optical isomers of BayK 8644 were examined; one isomer is inactive in both the calcium current and transmitter output assay. The other, R5417, increases calcium channels that are activated between -40 and zero millivolts, but increase transmitter output in only a small subset of neurons. This suggests that neurons may be heterogeneous with regard to the calcium channels involved in transmitter output and that channels in the cell body and synaptic terminals may have different properties. These data indicate that the pharmacology of transmitter release is complex and data regarding drug effects on calcium movements must be interpreted with caution.

### Excitatory amino acids and synaptic transmission

Drs. Mark Mayer and Gary Westbrook and their co-workers have been in the forefront of the extremely active area of research relating to mechanisms mediating excitatory synaptic transmission in the mammalian central nervous system. The acidic amino acids, L-glutamate and perhaps L-aspartate, are thought to be the transmitter substances signaling excitatory messages across central synapses, and are known to act at several subtypes of acidic amino acid receptors. Growing interest in this work within the neuroscience community reflects discoveries suggesting a role for excitatory amino acids in many diverse cellular functions; the areas attracting attention currently include the cellular basis of memory formation at synapses using L-glutamate as a transmitter; stabilization and elimination of synapses during development; the programming of motor activity; and cellular mechanisms underlying neuropathological processes including stroke and Alzheimer's disease. A basic understanding of the mechanisms of action of excitatory transmitters is fundamental to probing the cellular basis of these complex behaviors.

The NMDA subtype of glutamate receptor appears to participate in all of the above behaviors, reflecting three unique properties of the ion channels linked to NMDA-receptors: a relatively high permeability to calcium; voltage sensitivity due to channel block by magnesium; and regulation by complex modulatory mechanisms. Much of the past year's activity has been devoted to work on these three areas. Permeation studies have now provided an unambiguous characterization of the ionic selectivity of the

channels activated by excitatory amino acids; NMDA opens channels about 12 times more permeable to calcium than to sodium, while for both kainic and quisqualic acids, selective agonists for non-NMDA types of glutamate receptors, calcium is approximately 0.1 times as permeable as sodium. Block of NMDA receptors by Mg appears to reflect binding of Mg ions to a site within the channel. Permeant ions also appear to bind to this site, and the rate constants of ionic dehydration and binding of ions to side chain residues within the ion channel seem likely to account for the different behavior of Ca and Mg as permeators and blockers respectively. These rate constants span 4 orders of magnitude for Ca and Mg; Mn lies in the middle of this range and is both permeant, though less so than Ca, and a voltage-dependent blocker of weaker potency than Mg.

Modulation of NMDA receptor activity is emerging as an important regulatory mechanism governing excitatory synaptic transmission. Zinc is released from synaptic vesicles during transmission from mossy fiber afferents synapsing in area CA3 of the hippocampus; low concentrations of zinc, well within those believed to occur during synaptic transmission, selectively blocks responses to NMDA. The action of zinc shows noncompetitive kinetics, and does not vary with membrane potential in contrast to the action of Mg. Zinc also produces a small potentiation of responses to kainate and quisqualate. Submicromolar concentrations of glycine also regulate responses to NMDA, producing a profound potentiation of NMDA receptor activity. Current efforts are directed at exploring the mechanism of action of zinc.

Studies on synaptic transmission (I. Forsythe) provide a functional link between our biophysical experiments on the membrane action of L-glutamate and its analogues and the control of complex behaviors regulated by NMDA receptors. Until recently, fast excitatory synaptic transmission was thought to reflect the activity of L-glutamate acting at kainate and quisqualate receptors, and the role of NMDA receptors was unclear. It is now clear that activation of NMDA receptors during monosynaptic transmission contributes a small, but prolonged component to the synaptic response, and that this component is voltage dependent, associated with calcium influx, and modulated by glycine and zinc. Work is in progress to provide a more detailed picture of the release mechanisms governing activation of kainate/quisqualate and NMDA receptors at excitatory synapses.

#### Section on Neuroendocrinology

The Section on Neuroendocrinology, under the leadership of Dr. David C. Klein, has made significant progress in the general area of transmembrane signal transduction. The major program has focused on the question of how two types of receptors can interact in regulating cellular processes. This work comes from detailed studies on the rat pinealocyte. Workers in the Section have found that a two-receptor system controls both cyclic AMP



and cyclic GMP accumulation. One leg of this two-receptor system appears to act through GTP-binding regulatory proteins to activate adenylyl and guanylyl cyclases. This conclusion derives from work done on cyclic AMP by Dr. David Sugden and work on cyclic GMP done by Dr. Anthony Ho. This leg of the system is activated by  $\beta$ -adrenergic agonists. In addition, recent work by Constance Chik and Anthony Ho indicates that vasoactive intestinal peptide can substitute for  $\beta$ -adrenergic agonists. Although activation of this part of the system produces significant effects on both cyclic AMP and cyclic GMP, the responses represent a small fraction of the maximal response possible. The maximal response is elicited when the second leg of this system is activated.

Activation of the second leg is accomplished by activation of  $\alpha_1$ -adrenergic receptors. The pioneering efforts of A. Louise Sugden and David Sugden have shown that  $\alpha_1$ -adrenergic receptors activate processes which increase the concentration of calcium inside the cell, and that this increase in calcium is important for the increase in both cyclic AMP and cyclic GMP. Anthony Ho has discovered that  $\alpha_1$ -adrenergic receptors activate phospholipase C. This enzyme generates a lipid, diacylglycerol, which acts in concert with calcium to increase the activity of a special enzyme, a calcium-, phospholipid-dependent protein kinase, termed protein kinase C.

Anthony Ho has studied the regulation of protein kinase C in detail, and has found that the activation of the enzyme by adrenergic agonists is mediated by  $\alpha_1$ -adrenergic receptors. His associated studies indicate that the increase in intracellular calcium is of central importance in the transmitter activation of protein kinase C in intact pinealocytes, and that other agents which elevate calcium through different mechanisms have a similar effect on the enzyme. This work is of special importance in neurobiology because the pinealocyte is the only intact neural cell in which adrenergic activation of protein kinase C can be demonstrated. Adrenergic agonists and  $\alpha_1$ -adrenergic receptors are located throughout the nervous system. This makes results of studies on the pinealocyte of wide interest and of general importance.

The effects of protein kinase C appear to be at the level of the regulatory protein-catalytic protein interaction according to work by David Sugden and Anthony Ho. The enzyme might phosphorylate either protein. The net result is a marked increase in the efficiency of the activation of both adenylyl and guanylyl cyclase, according to work by Anthony Ho and Constance Chik. The two-receptor stimulation of cyclic GMP has an additional special requirement for calcium, according to work by Anthony Ho and Constance Chik. The precise role of calcium is not clear; it may be required for the  $\alpha_1$ -adrenergic activation of phospholipase A2 and for the production of key members of the arachidonic acid cascade.



These findings represent an important advance in the understanding of transmembrane signal processing since the mechanisms described could act as a neurochemical switch. The large responses in cyclic AMP or cyclic GMP which range from 100- to 600-fold, which are produced only when both receptors are activated, are clearly large enough to switch on cyclic AMP or cyclic GMP-sensitive ion channels or metabolic processes. However, activation of either receptor alone has little or no effect. Thus, this represents an excellent mechanism by which neural cells could integrate two transmitter inputs; only when both are active will the switch be activated and a third response will be produced.

Several new projects have been initiated in the Section. First, Jeri El Hage has started to study the important regulatory proteins which may be involved in the control of adenylyl and guanylyl cyclase, the GTP-binding protein. She has focused her attention on  $GS\alpha$  and has found that this protein is endogenously ribosylated. This is the first demonstration of endogenous ribosylation of  $GS\alpha$  in any intact cell system. It is important because studies with cholera toxin show that ribosylation of  $GS\alpha$  is sufficient to activate adenylyl and guanylyl cyclase. Thus this finding provides clear evidence that endogenous ribosylation of  $GS\alpha$  may be a physiological mechanism involved in the regulation of cyclases. El Hage has also found that factors which activate the pineal gland also change the capacity of  $GS\alpha$  to be ribosylated by cholera toxin, which provides further support for this hypothesis.

Another new effort has been conducted by Horst Korf, who has devoted his attention to characterizing the pineal cell population. He is intending to determine if the population consists primarily of one single group of cells, or if there are two major populations. He is conducting this study using radio labelled ligands and immunocytochemical tools.

The efforts of Joan Weller and M.A.A. Namboodiri are bringing the Section closer to cloning the gene for one of the most interesting enzymes in regulatory neurobiology, pineal serotonin N-acetyltransferase. Weller has now prepared sheep pineal cDNA libraries prepared from glands obtained at night and during the day. She intends to prepare a differential library from this and use this in conjunction with antiserum prepared by Namboodiri against highly purified preparation of night pineal glands containing N-acetyltransferase.

A fourth new area of activity is in the regulation of outward potassium currents. Valentine Cena has conducted biochemical studies which have shown that norepinephrine stimulates the efflux of potassium, and that this is mediated by  $\alpha_1$ -receptors in part, and that it appears to be due to the increase in intracellular calcium which activates a calcium-sensitive outward potassium channel. The existence of this channel has been documented in a collaborative effort with John Halperin, AFRI,

who has used patch clamp technology to study pineal membranes. His work has clearly identified a calcium-dependent outward potassium channel. This channel may be the one involved in the adrenergic stimulation of potassium efflux seen in intact cells.

The Section is increasing their activities in molecular neurobiology, with the appointment of a molecular geneticist, Randall McKinnon. The Section will obtain and use genetic probes of interest to study the expression of genes during development and as a function of neural regulation. Special emphasis will be given to factors involved in the regulation of the expression of pineal-specific genes and pineal-, retinal-specific genes.

#### Molecular Neurobiology Unit

The Molecular Neurobiology Unit has continued its work with differentiation-specific cDNAs from mouse neuroblastoma cells. Three cDNA clones have been identified which represent mRNAs of moderate to high abundance which are expressed only in cells grown on surfaces and hence may have some relationship to cell attachment or to the cytoskeleton. At least two of these sequences are expressed in brain and none of them is expressed in liver. There are complex relationships to RNAs in other cultured cell lines. The cDNA for approximately one-half of one of these mRNAs has been sequenced, and the other cDNAs have been restriction mapped in preparation for sequencing. Full-length cDNA libraries and genomic libraries have been prepared and are being screened for the two of these clones which are not full-length and for other sequences of neurobiologic interest.

Several cDNAs for mRNAs which are unique to, or enriched in, the subchronic reaction around a stab wound in rat cerebral cortex have been isolated and are being evaluated for their relationship to the wounded brain neurotrophic factor. These cDNAs were prepared from size-selected mRNAs which have been shown by oocyte translation to contain the coding sequence for that factor, and were selected by differential colony hybridization to control and wounded brain cDNAs. Fusion proteins expressed by the bacteria containing these cDNAs are being tested for their ability to enhance the survival of sympathetic ganglion neurons in culture.

Adult mammalian hypothalamus is a rich source of a peptide which is conserved from coelenterates through humans, a dodecamer called the Hydra head-forming peptide. Its function in mammalian brain is unknown, but its structural relationship to bradykinin and the presence of bradykinin receptors on mammalian glial cells and the absence of that peptide in mammalian brain suggest a possible role. We have obtained three cDNA clones from rat hypothalamus mRNAs with similar 1000 nucleotide inserts which hybridize to oligonucleotides that we designed to detect the sequences coding for the Hydra peptide. The sequence specific to that peptide is also being screened for in an RNA prep from Hydra.

A protein factor in the conditioned medium of cultured glial cells which stimulates the activity of the enzyme choline acetyltransferase in mouse spinal cord cell cultures has been partially characterized. Injection of RNA from these glial cells into Xenopus laevis oocytes resulted in the production of material with similar ChAT stimulating activity and the ability to increase significantly the number of morphologically identifiable ChAT-producing cells in cultures of rat septum. No activity of this material was found in a spinal cord cell survival assay. Presently, a variety of glial preparations is being evaluated as the best source of this factor, and glial RNA is being size-fractionated for injection into oocytes. The size class which contains the desired mRNA will be used to prepare cDNA clones for this factor.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00047-18 LDN

## PERIOD COVERED

October 1 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Studies of Neurons and other Cell Types

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Brenneman	Sr. Staff Fellow	LDN, NICHD
Others: R. Alderson	Staff Fellow	LDN, NICHD
D. Kniss	Prat Fellow	LDN, NICHD
D. Warren	Bio. Lab. Tech.	LDN, NICHD
T. Nicol	Lab. Aid	LDN, NICHD
E. Neale	Physiologist	LDN, NICHD
I. Forsythe	Visiting Fellow	LDN, NICHD
G. Westbrook	Sr. Staff Fellow	LDN, NICHD

## COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH (L. Eiden); Biological Psychiatry Branch, NIMH (C. Pert); University College, Cardiff (G. Foster).

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.6

## PROFESSIONAL:

2.3

## OTHER:

1.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Cell cultures from the fetal mammalian central nervous system were used to study the regulation of neurodevelopment by neuropeptides, trophic factors and electrical activity. Vasoactive intestinal peptide was shown to increase the number of astrocytes during development in culture. Radioligand binding studies on whole cell and membrane preparations of astrocytes indicated the presence of high affinity receptors for VIP. Inositol phospholipid turnover was shown to be increased in astrocytes after stimulation with norepinephrine and bradykinin, but not with VIP treatment. Viral pentapeptides (TTSYT and TTNYT) with sequence homology to VIP(7-11) were shown to exhibit VIP-like increases in neuronal survival during electrical blockade.

Feasibility studies using DEAE sephacel, affinity column (wheat germ agglutinin, Concanavalin A and heparin), and Amicon filtration indicated that an activity-related neuron survival factor and a choline acetyltransferase (CAT)-stimulating factor did retain biological activity through these separation procedures. A number of possible sources for the factors have been found and potential second messenger systems have been identified.

The survival of spinal cord neurons was shown to be influenced by NMDA antagonists. Activity blockade with TTX and the GABAergic agonist muscimol were shown to produce a reversible and developmentally sensitive decrease in the cells immunoreactive to methionine-enkephalin in spinal cord cultures.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00048-13 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transcription-level control of neurobiologic &amp; developmental phenomena

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.K. Schrier

Head

LDN, NICHD

## Others:

E. T. Butler, III Special Expert

LDN, NICHD

M. M. Voigt NRC-NSF Fellow

LDN, NICHD

T. T. Quach Visiting Fellow

LDN, NICHD

S. McCune NRSA Fellow

LDN, NICHD

R. Glatter Bio. Lab. Aid

LDN, NICHD

B. L. Judge ECU Coop Student

LDN, NICHD

## COOPERATING UNITS (if any)

Lab of Biochem. Genetics, NHLBI (M. Giovanni, D. Hilt, B. Raj-Amaladoss, H. Chin, M. Nirenberg); Neuropsychiatry Branch, NIMH (A-M. Duchemin, D-M. Chuang, R.J. Wyatt); Univ. of Texas Health Science Center at Dallas (L. Hersch); Dept. of Chem., California Institute of Tech. (F. Sutton)

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Molecular Neurobiology Unit

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS

3.5

## PROFESSIONAL

3.0

## OTHER

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Among thousands of clones selected from cDNA libraries of differentiation-regulated mRNAs from NS20Y neuroblastoma cells, we have selected three clones which are differentiation-specific. All of these are induced by growing the cells on a treated polystyrene surface, one is further stimulated by the addition of dibutyryl-cyclic AMP to the medium, two of them hybridize to transcripts in brain RNA, and two hybridize to a smaller size transcript from glioma cells and to a large and very abundant RNA in a human hepatoma cell line. (2) Several cDNAs for mRNAs which are unique to, or enriched in, the sub-chronic reaction around a stab wound in rat cerebral cortex have been isolated and are being evaluated for their relationship to the wounded brain neurotrophic factor. (3) Three cDNA clones from a rat hypothalamus library have been selected using synthetic oligonucleotide probes designed to detect the hydra head-forming peptide, a completely conserved dodecamer found in high concentrations in nervous tissues from coelenterates to man. These three clones have inserts of the same size, suggesting that they may represent full-length complements of the mRNA. (4) RNA preparations from cultured mouse glial cells have been injected into *Xenopus laevis* oocytes, and the translation products of these oocytes were active in stimulating the activity of the enzyme choline acetyltransferase in primary cultures of mouse fetal spinal cord or septum cells. Hence we have an assay for the enrichment of the factor encoding mRNA and for the screening of clones via hybrid selection methods.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00064-11 IDN

2

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Neurobiologic Studies of Neurons and Glia in Cell Culture

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	P.G. Nelson	Head	IDN, NICHD
------	-------------	------	------------

OTHERS:	C. Yu	Visiting Fellow	IDN, NICHD
	E. A. Neale	Physiologist	IDN, NICHD
	I. D. Forsythe	Visiting Fellow	IDN, NICHD

## COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH (J. Moskal)

## LAB/BRANCH

Laboratory of Developmental Neurology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.2

## PROFESSIONAL:

1.2

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A voltage-sensitive calcium channel agonist, BayK 8644, and its active isomer, R5417, increases calcium currents in spinal cord (SC) and dorsal root ganglion (DRG) neurons in cell culture under voltage clamp, particularly those currents which are activated between -40 and zero mV membrane potential. These agents do not increase excitatory transmitter output in this system, suggesting that the types of calcium channels sampled in the neuronal cell body may be distinct from those involved in transmitter release from neuronal terminals.

Physiological studies of synapse formation between neurons in different compartments of a 3-compartment culture system have begun in conjunction with morphological observations described in Project Z01 HD 00708-03. Chronic stimulation of DRG axons making synaptic connections to SC neurons appear to increase the relative efficacy of these stimulated synapses.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00094-17 LDN

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pineal Regulation: Environmental and Physiological Factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Klein	Head	LDN, NICHD
Other:	A.K. Ho	Visiting Fellow	LDN, NICHD
	H. Korf	Visiting Fellow	LDN, NICHD
	J. El Hage	IRTA	LDN, NICHD
	V. Cena	Guest Researcher	LDN, NICHD
	C. Gonzalez-Garcia	Guest Researcher	LDN, NICHD

COOPERATING UNITS (if any)

Cooperating Units: P. Skolnick, V. Cena, NIAMMD; D. Jacobowitz, S. Markey, NIMH; M.A.A. Namboodiri, Georgetown University, R. Janovsky, U. of Penn; K. Sweadner, Mass. Gen. Hospital

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.1

PROFESSIONAL

0.6

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the environmental and physiological regulation of the pineal gland, exclusive of transmembrane and intracellular regulatory mechanisms (See Z01-HD 00095-17 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. It has been proposed that the SCN pineal circuit passes through the paraventricular nucleus of the hypothalamus (PVN). This past year work was completed which supports this with the demonstration that electrical stimulation of PVN stimulated the production of melatonin at a near physiological rate. In other studies, the photoneural regulation of pineal rhodopsin kinase and phospholipase C have been studied; and the developmental appearance of both phospholipase C and Na<sup>+</sup>/K<sup>+</sup>-ATPase has been examined. It has been discovered that Na<sup>+</sup>/K<sup>+</sup>-ATPase develops after birth, as indicated by both ouabain binding and two indices of enzyme activity, ATP hydrolysis by membrane preparations and uptake of rubidium. Results indicate a high affinity form of Na<sup>+</sup>, K<sup>+</sup>-ATPase, similar to the α<sup>+</sup> form which has been described in the brain, is the dominant form present in the pineal gland. This indicates that another mechanism might generate membrane potential before this time.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00095-17 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Klein	Head	LDN, NICHD
Other:	A.K. Ho	Visiting Fellow	LDN, NICHD
	H. Korf	Visiting Fellow	LDN, NICHD
	J. El Hage	IRTA	LDN, NICHD
	V. Cena	Guest Researcher	LDN, NICHD
	C. Chik	Guest Researcher	LDN, NICHD
	J. Weller	Chemist	LDN, NICHD

## COOPERATING UNITS (if any)

W. Anderson, T.P. Thomas, NCI; M.A.A. Namboodiri, Georgetown U.; I. Gery, T. Shinohara, NEI; J. Halperin, AFRI

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neuroendocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.4

## PROFESSIONAL:

4.3

## OTHER:

1.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to discover the molecular basis of neurochemical transduction mechanisms, using the pineal gland as a model. Efforts are directed at determining the details of the chemical and ionic components of transmembrane signalling processing and in the neural regulation of gene expression. The most important advances made in the first area were those that have clearly indicated that cAMP and cGMP are regulated by a two receptor mechanism which appears to be focused on the regulation of adenylyl and guanylyl cyclases. One leg of this pathway activates these enzymes via GTP binding regulatory proteins, similar to G $\alpha$ . This leg is controlled by  $\beta$ -adrenergic or VIP receptors; activation of this leg produces only partial stimulation of cAMP and cGMP accumulation. Activation of the other leg is via  $\alpha$ 1-adrenergic receptors. This activates protein kinase C which acts, perhaps on the regulatory or catalytic proteins, to increase the activation of adenylyl and guanylyl cyclase. Activation of protein kinase C occurs as a result of an increase in [Ca $^{2+}$ ]<sub>i</sub> and in diacylglycerol production by phospholipase C. In addition, in the regulation cGMP, there appears to be a strong requirement for activation of phospholipase A and for an increase in [Ca $^{2+}$ ]<sub>i</sub>. In the area of the neural control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindole-O-methyltransferase, and in isolating cDNA clones coding for these enzymes.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00704-03 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Tetanus Toxin Effects and Localization in Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

Inactive



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00706-02 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Physiological Studies of Nervous System Development In Vitro

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER:

-

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Inactive

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00707-03 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Pharmacological Studies of Synaptic Transmission In Vitro

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.L. Mayer	Visiting Associate	LDN, NICHD
	G.L. Westbrook	Staff Fellow	LDN, NICHD

Others:	I.D. Forsythe	Visiting Fellow	LDN, NICHD
	P.G. Nelson	Head	LDN, NICHD
	K. Sugiyama	Visiting Fellow	LDN, NICHD

## COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NINCDS (J. Clements)

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

4.5

## PROFESSIONAL:

4.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Our experiments investigate the mechanism of action of excitatory amino acids as synaptic transmitters and neuromodulators in the vertebrate CNS, utilizing cell culture and electrophysiological techniques. Much of our work is on the N-methyl-D-aspartate (NMDA) receptor subtype. Permeation and block of NMDA receptor channels by divalent cations has been investigated using whole cell recording. Ba, Ca, Mn and Sr are all permeant, while Co, Mg and Ni are voltage dependent blockers. Reversal potential measurements with 0.1 to 50 mM extracellular Ca, and 50 to 150 mM extracellular Na, analysed using an extended constant field equation, show Ca to be approximately 10 times more permeant than Na. However binding of permeant divalent cations is suggested by their block of inward Na current, and alternative models are needed to describe permeation. Low concentrations of zinc and cadmium also block responses to NMDA, however their action is similar at +60 and -60 mV, and thus due to an action at a different site from that for Mg. Zinc acts as a noncompetitive NMDA receptor antagonist, and thus does not interfere with the initial binding of agonist. Fluctuation analysis shows a reduction in open time during Zn and Cd antagonism; possible models under consideration include ultra fast channel block and allosteric modulation to substates of reduced conductance and lifetime. Excitatory synaptic transmission in hippocampus and spinal cord has been studied under voltage clamp. Epsps are produced by two components of synaptic current: a fast inward current of decay time constant 1-5 ms due to activation of kainate/quisqualate receptors, and a slow component of time constant circa 80 ms due to activation of NMDA receptors. The slow component of the epsp is blocked by selective NMDA receptor antagonists. Including low concentrations of zinc, is voltage sensitive in the presence of Mg, and has a Ca-dependent reversal potential. Glycine (1  $\mu$ M) is a potent modulator of the slow epsp. Conditioned medium from hippocampal glial cell cultures also potentiates the slow epsp and responses to NMDA suggesting that release of modulatory substances from glial cells warrants further investigation. Analytical techniques developed to study the synaptic release process include deconvolution analysis, and a novel nonstationary fluctuation analysis of synaptic currents.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00708-03 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphologic studies of Neuronal and Non-neuronal Cells in CNS Cell Cultures

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Elaine A. Neale

Physiologist

LDN, NICHD

Others: P.G. Nelson

Head, SN

LDN, NICHD

C. Yu

Visiting Fellow

LDN, NICHD

L.M. Bowers

Biologist

LDN, NICHD

B.L. Judge

Co-op Student

LDN, NICHD

J.L. Koh

Bio-Aid

LDN, NICHD

## COOPERATING UNITS (if any)

Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration (W.H. Habig); Division of Pediatric Neurology, University of Minnesota (P.K. Sher); Department of Biochemistry, University of Texas (L.B. Hersch).

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Unit on Cell Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL:

1.8

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Morphologic techniques are applied to dissociated cell cultures of central nervous system. Tetanus toxin binding, visualized by immunohistochemistry, has proven an effective neuronal label within only a few hours of plating. Radioautography has shown that chronic exposure to benzodiazepine results in an apparent decrease in benzodiazepine receptors.

The methodology has been refined for the preparation of multicompartiment culture chambers to study the effects of neuronal activity on the formation, elimination, and stabilization of synaptic contacts. Reproducible survival of both input and target neurons is a minimal requirement for such studies. Reliable preparations have been achieved, and preliminary data indicate that chronic electrical stimulation is correlated with a relative increase in the number of stable synaptic contacts. Additional experiments indicate that stimulation confers no particular advantage in terms of neurite outgrowth, and may be somewhat detrimental in terms of neuron survival.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00709-01 LDN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of neuronal deficits associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Brenneman Staff Fellow LDN, NICHD

Others: G. Westbrook Staff Fellow LDN, NICHD

S. Fitzgerald Biologist LDN, NICHD

## COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH (C. Pert); Laboratory of Immunobiology, NCI (P. Nara, L. Arthur); Lab. of Microbiology & Immunology, NIDR (M. Ruff); Lab. of Microbial Immunity, NIAID (D. Ennist, K. Elkins).

## LAB/BRANCH

Laboratory of Developmental Neurology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.6

## PROFESSIONAL:

0.3

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell Cultures from the fetal mammalian central nervous system were used to study the neuronal death associated with Acquired Immune Deficiency Syndrome (AIDS). Purified envelope protein (gp120) from the AIDS virus was found to produce significant decreases in the number of surviving neurons in developing cultures derived from the spinal cord and hippocampus of the fetal mouse. Two characteristics of this toxicity were of particular interest. Gp120 produced neuronal deficits at extraordinarily low concentrations: 10<sup>-14</sup> M. Secondly, an attenuation of the neuron-depleting effects of gp120 were observed at concentrations greater than 10<sup>-11</sup> M.

Using dissociated hippocampal cultures as a model system, several substances were investigated for their effect on gp120-induced neuronal death. D-Ala-Peptide T-amide prevented gp120-related death in a dose-dependent manner. No apparent neuronal death was observed with the addition of 10<sup>-10</sup> M D-Ala-Peptide T-amide to gp120-treated test cultures. Vasoactive intestinal peptide, which contains a amino acid sequence similar to Peptide T, also prevented gp120-induced death at low (0.1 nM) concentrations.



LABORATORY OF DEVELOPMENTAL PHARMACOLOGY

Z01 HD 00136-19 Pharmacogenetics

Daniel W. Nebert, M.D.

Z01 HD 00503-03 Regulation and Expression of the UDP Glucuronosyl-  
transferase Gene Family

Peter I. Mackenzie, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1987

Laboratory of Developmental Pharmacology

SUMMARY

The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular mechanisms of gene expression involving drug-metabolizing enzymes. The clinical discipline involving the study of genetic differences in drug metabolism has been termed pharmacogenetics. Cytochromes P450 are enzymes involved in the oxidative metabolism of steroids, fatty acids, prostaglandins, leukotrienes, biogenic amines, pheromones, plant metabolites and bacterial cofactors. These enzymes also metabolize innumerable drugs, chemical carcinogens and mutagens, chemicals in foodstuff, and other environmental contaminants. The large degree of overlapping substrate specificities, classes of inducing agents, and drug-drug interactions have caused great difficulty in P450 studies at the level of catalytic activities and protein immunochemistry. P450 enzymes represent the classical "Phase I" metabolism in which the substrate is oxygenated. "Phase II" enzymes often use the oxygen as a site for further metabolism (e.g. glucuronidation, and sulfate, glutathione, or glycine conjugation). Detoxification usually requires both Phase I and Phase II enzymes.

Hundreds of drugs and other chemicals are known to stimulate (induce) their own metabolism or the metabolic fate of structurally-related compounds. In addition, steroids, prostaglandins, and small peptide hormones have been found to regulate some of these activities. The mechanisms surrounding the induction of these enzymes and expression of these genes are of central importance to fundamental molecular genetics, developmental biology, teratogenesis, carcinogenesis, mutagenesis, endocrinology, limnology, and drug addiction, tolerance and toxicity. This Laboratory presently comprises one Section and one Unit.

- A. The Section on Pharmacogenetics, under the direction of Daniel W. Nebert, M.D., is interested in the regulation and expression of genes encoding Phase I drug-metabolizing enzymes, most of which represent the P450 proteins, and certain Phase II drug-metabolizing enzymes. The P450 gene superfamily is presently known to comprise eleven P450 gene families, eight of which exist in mammals. Several conclusions about P450 gene evolution are apparent. The P450 superfamily is ancient and has expanded via divergent evolution. The ancestral P450 gene, present probably more than two and a half billion years ago, had a minimum of 40 exons. Estimates of the unit evolutionary period (UEP; millions of years required for 1% divergence in amino acid sequence) range between 5 and 6, but are difficult due to several instances of gene conversion between homologous P450 genes. Two mammalian mitochondrial P450 proteins, encoded by nuclear DNA, are more similar than the microsomal P450 proteins are to the prokaryotic P450 protein.

Striking differences in developmental-, sex- and tissue-specific P450 gene expression have been demonstrated by modern molecular biologic techniques. Furthermore, P450 expression vectors have recently been successfully transformed into yeast and transfected into mammalian cell cultures.

We have extensively studied the P450IA1 gene (trivial name, P<sub>1</sub>450) in mouse hepatoma Hepa-1 cultures and receptor-defective and P<sub>1</sub>450 metabolism-deficient mutant cell lines. Upstream P<sub>1</sub>450 regulatory sequences include: (i) the TATA box; (ii) a tetrachlorodibenzo-p-dioxin (TCDD)-inducible enhancer, which includes (iii) an element that augments constitutive gene expression; and (iv) a separate control element involved in a negative autoregulatory loop. Metabolism of substrate(s) by the product of the P<sub>1</sub>450 gene not only controls its own constitutive expression but regulates the expression of genes encoding at least two other enzymes having coordinate metabolic functions--UDP glucuronosyltransferase (UDPGT<sub>1</sub>) and NAD(P)H:menadiene oxidoreductase (NMOR<sub>1</sub>). The P<sub>1</sub>450, P<sub>3</sub>450, UDPGT<sub>1</sub>, and NMOR<sub>1</sub> genes have all been cloned, are under control of the aromatic hydrocarbon (Ah) receptor, and are defined as members of the [Ah] gene battery. Genes encoding the Ah receptor(s), the putative repressor, and other trans-acting regulatory factors are being cloned and characterized.

Projects in this Section are divided among (1) basic molecular biology and genetics, (2) evolution of these genes and regulatory regions, including studies involving DNA sequencing, chromosomal walking and mapping, and (3) clinically important applications. Experimental systems include the use of inbred mouse strains, transgenic mice, recombinant DNA technology, and somatic cell genetics in culture. As an example of a clinically important application, the human P<sub>1</sub>450 and P<sub>3</sub>450 genes and flanking regions have been cloned and sequenced, and localized near the MPI gene on chromosome 15. Evidence has been presented to suggest that human P<sub>1</sub>450 and P<sub>3</sub>450 genes, similar to their orthologues in laboratory animals, are important in the activation of inert chemical procarcinogens, promutagens and proteratogens to active metabolites. Restriction fragment length polymorphisms (RFLPs) have been found, and families with high and low cancer incidence are being studied. In the future it should be possible to correlate RFLP patterns of these genes with human disease. Such tests would facilitate the evaluation of cancer and toxicity risk for individuals exposed to foreign chemicals. These assays would aid the individual, employer and physician in decisions regarding life style, cigarette smoking, employment, and prescription drugs.

- B. The Unit on Recombinant DNA and the Conjugating Enzymes, under the direction of Peter I. Mackenzie, Ph.D., studies the regulation and expression of several subfamilies of the rat UDP glucuronosyltransferase (UDPGT) gene family. The function of the UDPGT enzymes is to conjugate oxygenated (or N- or S-containing) metabolites with glucuronic acid, thereby rendering the glucuronide conjugate extremely hydrophilic and, hence, detoxified and readily excreted. It follows logically that, if P450 oxygenates a hydrophobic drug or other chemical (Phase I metabolism) and UDPGT conjugates the oxygenated intermediate (Phase II metabolism), the two gene systems might be under some sort of coordinate regulation. Interestingly, UDPGT enzymes are similar to P450 enzymes in that (i) some genes are expressed constitutively and (ii) others are inducible by (a) combustion products such as benzpyrene and TCDD, (b) phenobarbital, (c) steroids or (d) clofibrate peroxisome proliferators such as clofibrate.

The isolation and sequencing of seven cDNA clones has demonstrated the existence of at least four different forms of transferase belonging to two gene subfamilies. UDPGT<sub>r</sub>-2, a phenobarbital-inducible isozyme, has 529



amino acids and is active in the glucuronidation of testosterone, dihydrotestosterone, estradiol and the foreign compounds chloramphenicol, 4-hydroxybiphenyl and 4-methylumbelliferone. The amino acid sequence of UDPGT-2 is about 65% similar in sequence to the other forms. UDPGT<sub>r</sub>-3 and UDPGT<sub>r</sub>-5 also have activity toward testosterone, dihydrotestosterone and  $\beta$ -estradiol whereas UDPGT<sub>r</sub>-4 preferentially uses etiocholanolone, androsterone and lithocholic acid as substrates. The latter three cDNAs are greater than 85% similar in sequence and the levels of their mRNA counterparts appear not to be increased by either phenobarbital or 3-methylcholanthrene. Homologous genes of this subfamily are also found in the mouse, where they are localized on chromosome 5. The mRNAs of all four forms of UDPGT are highest in the liver compared to intestine, kidney, lung and testis and are elevated postnatally. Although differing in primary amino acid sequence and the number of potential glycosylation sites, all forms have structural motifs in common including a cleavable amino-terminal signal sequence and a carboxy terminus composed of a stretch of 17 hydrophobic residues followed by a highly basic region of about 20 amino acids. These data suggest that the active sites of these enzymes are on the luminal side of the endoplasmic reticulum. Genomic clones to UDPGT<sub>r</sub>-4 have been isolated and are being sequenced. Genomic and cDNA clones have been utilized in an ongoing study of the regulation of each form as a function of age, tissue distribution and administration of prototypic inducers.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00136-19 LDP
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) PHARMACOGENETICS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D. W. Nebert	Head LDP, NICHD
Others:	See ATTACHMENT I	
COOPERATING UNITS (if any)  See ATTACHMENT II		
LAB/BRANCH Laboratory of Developmental Pharmacology		
SECTION Section on Pharmacogenetics		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
8.6	6.5	2.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The cytochrome P450 gene superfamily is known to contain at least eleven gene families and most likely many more. Eight of these families exist in all mammals. This laboratory has studied most extensively the tetrachlorodibenzo-p-dioxin (TCDD; in the lay press called "dioxin")-inducible P450I gene family, which has two members, P450IA1 and P450IA2, trivial names P<sub>1</sub>450 and P<sub>3</sub>450, respectively. We have examined the P<sub>1</sub> gene (P450IA1) in mouse hepatoma Hepa-1 cultures and receptor-defective and P<sub>1</sub> metabolism-deficient mutant cell lines. Upstream P<sub>1</sub> regulatory sequences include: (a) the TATA box; (b) a TCDD-inducible enhancer, which includes (c) an element that augments constitutive gene expression; and (d) a separate control element involved in a negative autoregulatory loop. The negative regulatory element involved in derepression of constitutive transcription, as well as the TCDD-inducible enhancer, appear to require a functional <u>aromatic hydrocarbon</u> (Ah) receptor. Metabolism of substrate(s) by the product of the P<sub>1</sub> gene not only controls its own constitutive expression but also regulates the activities of at least two other enzymes having coordinate metabolic functions--UDP <u>glucuronosyltransferase</u> (UDPGT<sub>1</sub>) and <u>NAD(P)H:menadione oxidoreductase</u> (NMOR<sub>1</sub>). The P<sub>1</sub>, P<sub>3</sub>, UDPGT, and NMOR<sub>1</sub> genes (which we have cloned) are all under control of the Ah receptor and are defined as members of the [Ah] gene battery. The Ah receptor is postulated to comprise a TCDD-binding subunit encoded by Gene B and a chromatin-binding subunit encoded by Gene C. The negative control element interacts with a P<sub>1</sub> metabolism-dependent repressor encoded by Gene N. We intend to clone and characterize all three of these genes encoding <u>trans-acting</u> factors. One long-range goal of this laboratory is to develop assays, based on recombinant DNA technology, to assess the human <u>Ah</u> phenotype and other pharmacogenetic disorders. Such assays may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.           </p>		

## ATTACHMENT I - Others:

Cheryl L. Butler	Biologist (Tech.)	LDP	NICHD
Peter C. Calafiura	Guest Researcher	LDP	NICHD
Cynthia A. Edwards	Staff Fellow	LDP	NICHD
Rene Feyereisen	Guest Researcher	LDP	NICHD
Josette Feyereisen-Koener	Guest Researcher	LDP	NICHD
Saikh J. Haque	Visiting Fellow	LDP	NICHD
Kiyoko Ikeya	Visiting Fellow	LDP	NICHD
Anil K. Jaiswal	Visiting Associate	LDP	NICHD
John E. Jones	Guest Researcher	LDP	NICHD
Kristi L. Kotz	Federal Junior Fellow	LDP	NICHD
Jong-Youn Lee	Visiting Fellow	LDP	NICHD
Peter I. Mackenzie	Visiting Scientist	LDP	NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP	NICHD
Roland A. Owens	Guest Researcher	LDP	NICHD
W. Vincent Picolo	Clinical Staff Fellow	LDP	NICHD
Alvaro Puga	NIH Expert	LDP	NICHD
Vesna Rapić	Guest Researcher	LDP	NICHD
Baisakhi Raychaudhuri	Visiting Fellow	LDP	NICHD
Kalman F. Salata	Staff Fellow	LDP	NICHD
Yhun Y. Sheen	Visiting Fellow	LDP	NICHD
Hana H. Smith	Chemist (Tech.)	LDP	NICHD
Rosalind J. Welty	Guest Researcher	LDP	NICHD



## ATTACHMENT II - COOPERATING UNITS:

H. Autrup, The Fibiger Institute, Laboratory of Environmental Carcinogenesis, Ndr. Frihavnsgade 70, DK-2100 Copenhagen Ø, Denmark

K. Berg, Institute of Medical Genetics, University of Oslo, Blindern, Oslo, Norway

A.-L. Børresen, The Norwegian Radium Hospital, Institute for Cancer Research, Department of Genetics, Montebello 0310, Ullernchausseen 70, Oslo 3, Norway

B. Brooks, Biochemistry Department, St. Mary's Hospital Medical School, University of London, London W2, England

H.-C. Chen, Endocrinology & Reproduction Research Branch, NICHD, NIH, Bethesda, Maryland 20892

R. R. Cobb, Department of Chemistry & Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709

F. J. Gonzalez, Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20892

J.-L. Guenet, Institut Pasteur, 28, Rue Du Dr Roux, 75724 Paris Cedex 15, France

O. Hankinson, Laboratory of Biomedical & Environmental Sciences, UCLA, 900 Veteran Avenue, Los Angeles, California 90024

K. Henning, Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

H. Hoffman, Animal Genetic Systems, Inc., 628-G Lofstrand Lane, Rockville, Maryland 20850

R. E. Kouri, BIOS Corporation, 291 Whitney Avenue, New Haven, Connecticut 06511

C. Kozak, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland 20892

R. Lindahl, Department of Biology, University of Alabama, P.O. Box 1927, Tuscaloosa, Alabama 35487

O. W. McBride, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20892

U. Meyer, Department of Pharmacology, Biozentrum, Basel, Switzerland

W. L. Miller, Department of Pediatrics, University of California, School of Medicine, San Francisco, California 94143

E. R. Simpson, Department of Biochemistry & Ob./Gyn., University of Texas Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235

J. von Borstel, Department of Genetics, University of Alberta, G216 Biological Sciences Centre, Edmonton T6G 2E9, Canada

M. R. Waterman, Department of Biochemistry, University of Texas, Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235

W. W. Weber, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104

H. Westphal, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, Maryland 20892

P. White, Cornell University Medical College, Division of Pediatric Endocrinology, 525 East 68th Street, New York, New York 10021

D. Wu, Department of Tumor Research, Fujian Medical College, Central 817 Road, Fuzhou, Fujian, China

H. Yonekawa, Department of Biochemistry, Saitama Cancer Center Research Institute, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00503-03 LDP
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) REGULATION AND EXPRESSION OF THE UDP GLUCURONOSYLTRANSFERASE GENE FAMILY		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	P. I. Mackenzie	Head LDP, NICHD
Others:	See ATTACHMENT I	
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Pharmacology		
SECTION Unit on Recombinant DNA and The Conjugating Enzymes		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)		
<p>The molecular mechanisms governing the regulation of the drug-detoxifying enzyme, UDP glucuronosyltransferase (transferase), and the structural differences between members of this family are being investigated in the rat. This animal, as exemplified by the Gunn rat, provides the only known animal model for investigating the defect in the glucuronidation of bilirubin and certain xenobiotics, characteristic of the Crigler-Najjar syndrome in humans. Certain strains of Wistar rat also have an inherited defect in the glucuronidation of steroid hormones. The isolation and sequencing of seven cDNA clones has demonstrated the existence of at least four different forms of transferase belonging to two gene subfamilies. Homologous genes of one of these subfamilies is located on mouse chromosome 5. All the forms have structural motifs in common, including a cleavable signal peptide and a carboxy-terminal transmembrane segment, which suggests that their active sites are on the luminal aspect of the endoplasmic reticulum. Expression of cDNAs in monkey COS cells demonstrated that three clones pUDPGT<sub>r</sub>-2, 3 and 5 encode transferases which glucuronidate testosterone and dehydrotestosterone. In addition, UDPGT<sub>r</sub>-2 is also active towards foreign chemicals. UDPGT<sub>r</sub>-4, however, is more active towards the 3-hydroxy position of the androgens, androsterone and etiocholanolone. Genomic clones to UDPGT<sub>r</sub>-4 have been isolated and are being sequenced. cDNA clones have been utilized to study the regulation of each form as a function of age, tissue distribution and administration of prototypic inducers.</p>		



## ATTACHMENT I - Others:

Cheryl L. Butler	Biologist (Tech.)	LDP	NICHD
Peter C. Calafiura	Guest Researcher	LDP	NICHD
Cynthia A. Edwards	Staff Fellow	LDP	NICHD
Saikh J. Haque	Visiting Fellow	LDP	NICHD
Kiyoko Ikeya	Visiting Fellow	LDP	NICHD
Anil K. Jaiswal	Visiting Associate	LDP	NICHD
John E. Jones	Guest Researcher	LDP	NICHD
Kristi L. Kotz	Federal Junior Fellow	LDP	NICHD
Jong-Youn Lee	Visiting Fellow	LDP	NICHD
Daniel W. Nebert	Chief	LDP	NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP	NICHD
Roland A. Owens	Guest Researcher	LDP	NICHD
W. Vincent Picolo	Clinical Staff Fellow	LDP	NICHD
Alvaro Puga	NIH Expert	LDP	NICHD
Vesna Rapić	Guest Researcher	LDP	NICHD
Baisakhi Raychaudhuri	Visiting Fellow	LDP	NICHD
Kalman F. Salata	Staff Fellow	LDP	NICHD
Yhun Y. Sheen	Visiting Fellow	LDP	NICHD
Hana H. Smith	Chemist (Tech.)	LDP	NICHD
Rosalind J. Welty	Guest Researcher	LDP	NICHD



# LABORATORY OF MOLECULAR GENETICS

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Michael Cashel, M.D., Ph.D.
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Igor B. Dawid, Ph.D.
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Xenopus Laevis  
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- Z01 HD 01004-04 Regulation of Amino Acid Biosynthetic Genes in  
Saccharomyces Cerevisiae  
Alan G. Hinnebusch, Ph.D.





NICHD Annual Report  
October 1, 1986 to September 30, 1986

Laboratory of Molecular Genetics

Recombinant DNA technology opened a new chapter in biology, especially in the biology of eukaryotic organisms. The ability to isolate and study in detail individual genes from complex genomes made possible entirely new types of analysis, allowing rapid progress in many areas notably including the area of developmental biology. Yet, isolating and characterizing genes is only one side of the technology needed to understand their biological function; it is also necessary to reintroduce isolated genes into the living organism so that function can be studied. Methods allowing such reintroduction have been crucial for progress in biology over the past three decades. The feasibility of moving genes into bacterial cells by conjugation, transduction and, later, calcium-mediated uptake of DNA, was critical in advancing molecular biology overall and constitutes a cornerstone in the development of recombinant DNA technology. About ten years ago techniques have been devised for transforming cultured cells, and for introducing genes into yeast, both of which had important applications. Yet, developmental biology studies more complex organisms - although single celled organisms do undergo certain aspects of development - and therefore it was particularly important to devise procedures that would allow effective introduction of isolated genes into higher animals. Techniques that allow introduction of genes into the germ line and their permanent transmission are now available in several cases, most notably for *Drosophila* with the aid of the P element vectors, and in the mouse by direct injection into a pronucleus of the one-cell embryo. In addition, genes can be introduced into different cells, most usefully oocytes or fertilized eggs of amphibians and sea urchins, and assayed for function in the immediately succeeding period in what is called a transient assay. During the past year different research groups in the Laboratory have utilized these techniques in a variety of organisms with the aim to further understanding of the molecular genetic and developmental aspect of gene function.

Heiner Westphal and his colleagues have applied the gene transfer technology in the mouse, generating transgenic animals that carry a variety of new information in their germ line. In previous years this group, in collaboration with Joram Piatigorsky and colleagues of the Eye Institute, showed that a short region from the  $\alpha A$  crystallin gene could direct expression of a transgene exclusively to the lens fiber cells where crystallins are expressed normally. Subsequently, these workers utilized the crystallin promoter/enhancer segment to direct the expression of an oncogene, the T antigen of SV40, to the eye, resulting in the highly reproducible generation of lens tumors in the transgenic animals. This result is remarkable because spontaneous tumors of the lens are entirely unknown. Thus, the lens is not inherently refractory to tumor growth. Neoplastic conversion was observed immediately after day 12 of development when the crystallin-promoted T antigen is first expressed in the embryo. During this period, the same cells often express T antigen and crystallins at the same time, allowing interesting future studies on the relationship between tumorigenesis and differentiation.

The protooncogene *c-mos* under the control of the long terminal repeat (LTR) of the Moloney murine sarcoma virus was introduced into mice in a collaboration with the laboratory of George Vande Woude. While this LTR/*c-mos* construct effectively transforms 3T3 cells in culture its expression in the transgenic mouse did not

generate tumors. Instead, transgenic animals showed an inhibition of differentiation of lens fiber cells, leading to lens swelling and disorganization. The basis for this interference with lens differentiation is under study, but it is already apparent that an activated protooncogene that has powerful transforming ability in cultured cells is not necessarily oncogenic in the intact animal.

A third application of the transgenic technology concerns the study of certain aspects of the biology of HIV, the causative agent of AIDS. In a collaboration with Malcolm Martin and colleagues, mice carrying the LTR of HIV linked to the reporter gene CAT, were found to express low levels of CAT primarily in the thymus. This expression could be stimulated by mitogen treatment or by infection with adenovirus or cytomegalovirus. The parallels between HIV induction in T<sub>4</sub> lymphocytes of AIDS patients and CAT induction in these transgenic mice suggest that the mice constitute a useful model system for the study of factors that affect HIV expression and the onset of AIDS disease in infected individuals.

A distinct project, in collaboration with Peter Gruss, concerned the spatial regulation of expression of the Hox1.1 homeobox gene in the mouse embryo. The known importance in *Drosophila* development of genes containing the homeobox, and the high degree of conservation of this sequence, suggest that homeobox-containing genes are developmental regulatory genes in other organisms as well. In situ hybridization experiments showed Hox1.1 expression in a localized region of somites in the early embryo; during subsequent development expression becomes limited to the sclerotomes while being turned off in dermatomes and myotomes. This complex developmental behavior is consistent with but does not establish a regulatory role for the Hox1.1 gene in development.

Igor Dawid, Tom Sargent and their colleagues have continued their studies on different aspects of gene expression in the amphibian embryo. One aspect of the program that uses gene transfer techniques, involves the study of the regulation of epidermal keratin expression. In past years several keratin genes have been characterized that are expressed in the *Xenopus* embryo. These genes are activated in a cell autonomous fashion (i.e., independent of cell interactions) during late blastula, exclusively in the animal region which subsequently will form the ectoderm. Because of this regionally specific and cell autonomous activation it appears likely that the factor(s) responsible for this activation is already localized in the animal region of the fertilized egg. Such a factor could be considered a carrier of cytoplasmic developmental information, suggested by embryologists as a basis for embryonic differentiation since the 19th century. To approach this issue a modified keratin gene was injected into fertilized *Xenopus* eggs and its activity assayed at different stages of development over the subsequent two days. Good evidence for tissue specific expression of such injected constructs has been obtained, opening the way to a detailed analysis of the DNA regions required for this activation (promoter/enhancer studies), and eventually analysis of protein factors binding to and affecting the function of these regions.

A second aspect of this work concerns the identification of new marker genes that are expressed in a tissue-specific way in embryogenesis. In the past year a notochord-specific keratin gene has been isolated and characterized. The notochord is important as the most dorsal mesodermal derivative in the embryo and a major element in the formation of the dorsal axis. In addition, genes that appear to be specific for early neural development, and for a head structure called the cement gland, have been isolated. These genes are being characterized further and promise to become useful tools in the molecular dissection of early tissue differentiation in



the amphibian embryo.

A third aspect of great interest in the laboratory is the analysis of mesoderm induction. Earlier studies have suggested that mesodermal derivatives arise from cells that receive a signal during cleavage and blastula stages originating in vegetal (future endodermal) cells. Recently, Jim Smith in London discovered a cell line, called XTC, that produces a factor that effects mesoderm induction. It was found in this laboratory that XTC medium not only induces the mesodermal marker  $\alpha$ -actin but also suppresses the ectodermal marker, epidermal keratin. Fibroblast growth factor (FGF), especially in combination with tumor growth factor  $\beta$  (TGF- $\beta$ ), has mesoderm inducing capacity; but it is not clear whether the XTC factor(s) is actually FGF and TGF- $\beta$ , or whether any of these factors act in the embryo itself. Future work in the laboratory is directed towards a resolution of this question and towards a molecular analysis of the action of mesoderm inducing factors, especially their effect on the expression of tissue-specific genes in the embryo.

Developmental genetics of *Drosophila* has been studied by a group headed by Igor Dawid. The locus *fs(1)h* has a maternal effect, i.e., a product of this locus layed down by the mother in the oocyte is required for normal development of the progeny. The *fs(1)h* locus interacts with other homeotic loci, notably *trithorax* (*trx*) and Ultrabithorax (*Ubx*), to specify normal segment identity in the fly. The *fs(1)h* locus has been cloned as reported previously. In the present year the sequence of the major ovarian transcripts has been determined, and antibodies have been prepared against the predicted protein. The major conclusions from the use of these antibodies is: (i) The *fs(1)h* protein is uniformly distributed in the embryo in spite of the regionally specific actions of the locus, and (ii) the *fs(1)h* protein is most likely a membrane glycoprotein.

The *trithorax* locus is of interest because of its interactions with *fs(1)h* but even more in its own right as a regulatory homeotic locus that is required from normal function of bithorax and Antennapedia complex genes. The *trx* locus has been cloned with the aid of P element-containing mutants. A very large transcript, >10 kb, has been detected by RNA blot hybridization with *trx* genomic sequences. Ongoing work aims to determine the limits of the gene, to characterize the molecular nature of the transcript(s), and to study the temporal and spatial regulation of *trx* expression.

Judith Levin and her colleagues have continued their studies on the retrovirus pol gene, focusing on correlation of genetic structure with pol-associated enzymatic functions. An MuLV reverse transcriptase clone was expressed in *E. coli*. The product, which differs from the viral enzyme in only 5 amino acids at the N terminus and by 14 amino acids at the C terminus, has normal polymerase activity, but much lower levels of RNase H activity than are associated with the viral enzyme. These results are consistent with the earlier prediction by this group that polymerase activity is localized to the N-terminal domain of reverse transcriptase and further suggest that the active site for RNase H is associated with the C-terminal region of the enzyme. Monospecific antibodies prepared against the bacterially-expressed reverse transcriptase have assisted in studying the nature of a previously recognized complex between viral reverse transcriptase and endonuclease. The complex does not involve disulfide bonds and thus appears to be entirely non-covalent.

Antibodies are also being used to elucidate relationships between Moloney MuLV and AKR reverse transcriptases and endonucleases. Additional reagents, preferably

monoclonal antibodies, are being prepared for continuation of this project.

Alan Hinnebusch and his colleagues have continued their studies in yeast molecular genetics, focusing on general control of formation of amino acid biosynthetic enzymes by amino acid availability in the growth medium. Earlier studies have shown that a hierarchy of genes affect this system. The proximal regulatory gene is *GCN4* whose product is an activator of many genes of amino acid biosynthetic pathways. *GCN4* expression is regulated at the translational level, mediated by 4 short open reading frames (ORFs) in the 5' segment of the mRNA; genes upstream in the hierarchy of general control affect this translational regulation. In the present reporting period the sequences in *GCN4* required for regulation were defined in further detail. A 240 bp segment containing all 4 ORFs and corresponding to about one-third of the 5' untranslated region of the mRNA, proved sufficient to confer complete regulatory behaviour on a heterologous transcript. However, more than the 4 ORFs alone is required: deletion of sequences between them destroyed regulation. Further it could be shown that the termination codon of ORF 4 is dispensible for regulation, but termination at ORF 1 is not. Further studies are aimed at elucidating the contributions of initiation and termination at each of the ORFs to regulation of *GCN4* expression.

Regulation of *GCN4* expression is mediated by upstream loci named *GCN* or *GCD* loci, depending on their positive or negative regulatory effects. Since *GCN4* regulation occurs at the translational level it is expected that some of the other loci may be involved generally in protein synthesis. Evidence favoring this hypothesis has been obtained in studies of some of the other *GCN* and *GCD* genes. Mutations in *GCD* genes are pleiotropic; they are temperature sensitive lethals, and have increased sensitivity to certain antibiotics that are inhibitors of protein synthesis. Further, some *GCD* mutants show a decreased level of misreading compared to wild type yeast. These results support the idea of *GCD* gene involvement in protein synthesis. A cell free protein synthesis system is being adapted for the purpose of studying the phenomena in more detail. For further detailed analysis of individual genes the *GCD12* gene has been cloned and its location mapped to chromosome VII. Studies on the positive regulator *GCN3* have shown that the gene product is expressed constitutively, thus implying that activation of its regulatory function in starvation conditions occurs post-translationally.

The strengths of yeast as an experimental system also figures in the work of Robert Crouch and his colleagues. The interests of this group are focused on RNA processing at the enzymological level; here, particular emphasis has been placed on RNase H. This enzyme has been suspected to be important in cell metabolism because of its presence in all organisms studied, but direct evidence for its function in higher organisms had been lacking. In recent years this research group isolated the RNase H genes from *E. coli* and from yeast. Gene disruption and overexpression studies in *E. coli*, begun earlier and continued in the present reporting period, showed that RNase H is involved in repair and probably replication. In yeast, where there are several RNase H genes, inactivation of one of them (*RNH-1*) does not result in any phenotype.

Another project pursued during this year concerns the *rrp-1* protein of yeast which is known to be involved in the production of 5.8S and 28S rRNA. The gene has been cloned and sequenced; it does not appear to encode a ribosomal protein. Further studies are directed at elucidation of the function of *RRP-1* in rRNA processing.

Michael Cashel and his colleagues have continued their studies of global control of metabolism in *E. coli*. The question asked is by what mechanism the expression of many different genes is regulated in the cell in response to nutrient availability and other external factors. Special attention is given to the effects of the nucleotide guanosine 3', 5'-bispyrophosphate (ppGpp), a known regulator of gene activity in bacteria. In the current reporting period attention focused on enzymes that are responsible for ppGpp production and degradation with the aim to vary at will the ppGpp concentrations in the cell, and to gain understanding how the concentration of this regulatory molecule is itself regulated.

Two genes concerned with ppGpp metabolism have been characterized: The *relA* gene, which encodes an enzyme that catalyzes synthesis of ppGpp on the ribosome in response to protein synthesis stalled for lack of aminoacylated tRNA, and the *spoT* gene, which encodes a ppGpp 3'pyrophosphohydrolase that is the main agent of cellular ppGpp degradation. The *spoT* gene has been mutationally altered to varying degrees such that a range of ppGpp basal levels could be obtained during steady state growth; assays of transcription in these strains allowed assessment of the role of ppGpp levels independently of nutritional state. The results indicate a likely regulatory role for ppGpp during normal growth in addition to the dramatic changes in concentration during physiological stress, a condition that had been the primary focus of earlier studies. The *spoT* gene itself has been the subject of detailed study; it has been identified as an internal open reading frame within a multicistronic operon, about 1 Kb away from its promoter. Fusions of *spoT* operon transcripts suggest negative regulation by ppGpp.

The other gene important in ppGpp metabolism, *relA*, has also been analyzed. In collaboration with Dr. G. Glaser (Jerusalem), the *relA* gene has been localized in a monocistronic operon; fusions of its promoter to a lac reporter gene also indicate inhibition by ppGpp. The *relA* and the *spoT* gene sequences share considerable (40% identity) homology; this is an intriguing result since the two genes, while concerned with metabolism of the same regulatory substance, encode two proteins with quite distinct enzymological functions.

Sequence verified null mutations in the *relA* gene and the *spoT* gene have been prepared. The *relA* null allele, while showing a more severe phenotype than previously known *relA* mutations, is viable, demonstrating that the *relA* gene is nonessential, and that an alternative pathway for ppGpp production must exist. The *spoT* null allele behavior shows that previous *spoT* mutations were partially active and that deletion of the gene is lethal, probably because of the inhibitory effects of the ensuing high levels of ppGpp. The lethality of the *spoT* null allele in haploids can be reversed by suppressor mutations, which probably lower ppGpp levels either by inhibiting alternative routes of ppGpp synthesis or by stimulating alternative routes of degradation. Two such mutations have been isolated; one is located adjacent to the *spoT* locus and the other does not map near either the *relA* or *spoT* genes.

Robert Welsberg and his colleagues have continued their studies on lambdoid bacteriophages, focusing both on regulation of transcription and on the mechanism of recombination.

A particularly interesting set of observations emerged from comparative studies that use phage HK022 in addition to the traditional object, phage  $\lambda$ . A new transcription termination factor with a remarkable specificity has been characterized: the Nun protein of phage HK022. Nun converts the phage  $\lambda$  *nut* sites, which, in the



presence of the  $\lambda$  N protein, are required for transcription antitermination, into transcription terminators. The relative amount of Nun and N determines whether termination or antitermination predominates at *nul*. Genetic studies suggest that Nun, like N, interacts directly with *nul* site transcripts, and that productive interaction requires a set of host encoded proteins called Nus factors. Sequencing and overexpression of the *nun* gene reveals that Nun is a small basic protein whose amino acid sequence is distantly related to that of N. Moreover, the location and orientation of *nun* in the HK022 genome resembles that of gene N in  $\lambda$ . However, in contrast to the essential role of N in  $\lambda$  growth, Nun does not stimulate HK022 growth. Therefore it is suggested that its principal biological role is exclusion of competing  $\lambda$ .

Studies on genetic recombination also benefitted from the comparison between  $\lambda$  and HK022. Both phages encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, the primary structure of the HK022 attachment sites and recombination proteins has been determined and compared to the analogous  $\lambda$  elements. The comparison shows that similarities and differences are interspersed within the HK022 attachment site and within the carboxyl-terminal three-fourths of Int-HK022; the amino-terminal one-fourth of Int-HK022 and the entire sequence of Xis-HK022, the other phage-encoded recombination protein, are identical to their  $\lambda$  analogues.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00066-17-LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control Mechanisms in Temperate Bacteriophage Lambda

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert Weisberg	Head	
Others:	Jeff Baron	Medical Staff Fellow	LMG, NICHD
	Kaymeuang Cam	Visiting Fellow	LMG, NICHD
	Naomi Kislev	Guest Worker	LMG, NICHD
	Nagaraja Ramaiah	IRTA	LMG, NICHD
	Sieghild Sloan	Microbiologist	LMG, NICHD
	Ezra Yagil	Visiting Scientist	LMG, NICHD

## COOPERATING UNITS (if any)

Institute of Cancer Research; Columbia University, NY, NY (Drs. Max Gottesman and Bernard de Massy); Laboratory of Molecular Biology; NIMH (Dr. Howard Nash); Genentech; South San Francisco, CA (Dr. Harvey Miller)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Microbial Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.9

## PROFESSIONAL:

2.9

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have characterized a new transcription termination factor with a remarkable specificity: the Nun protein of phage HK022. Nun converts the phage  $\lambda$  *nut* sites, which, in the presence of the  $\lambda$  N protein, are required for transcription antitermination, into transcription terminators. The relative amount of Nun and N determines if termination or antitermination predominates at *nut*. Genetic studies suggest that Nun, like N, interacts directly with *nut* site transcripts, and that productive interaction requires a set of host encoded proteins called Nus factors. Sequencing and overexpression of the *nun* gene reveals that Nun is a small basic protein whose amino acid sequence is distantly related to that of N. Moreover, the location and orientation of *nun* in the HK022 genome resembles that of gene N in  $\lambda$ . However, in contrast to the essential role of N in  $\lambda$  growth, Nun does not stimulate HK022 growth. We therefore suggest that its principal biological role is exclusion of competing  $\lambda$ .

HK022 and  $\lambda$  both encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, we have determined the primary structure of the HK022 attachment sites and recombination proteins, and have compared them to the analogous  $\lambda$  elements. The comparison shows that similarities and differences are interspersed within the HK022 attachment site and within the carboxyl-terminal three-fourths of Int-HK022; the amino-terminal one-fourth of Int-HK022 and the entire sequence of Xis-HK022, the other phage-encoded recombination protein, are identical to their  $\lambda$  analogues.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00067-19 LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Integration of Macromolecular Synthesis in *E. coli*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. C. Michael Cashel Head  
Others: Kenneth E. Rudd Staff Fellow Miklos Kalman  
Edoardo Sarubbi Visiting Fellow  
Shaw Chen Medical Staff Fellow  
Sharon Zemel Medical Staff Fellow  
Chikh Bengra Visiting Fellow  
Hua Xiao Visiting Fellow  
Kenji Ikehara Guest Worker

## COOPERATING UNITS (if any)

Dr. Gad Glaser: Dept. Cellular Biochemistry  
Hadassah Medical School  
Jerusalem, Israel

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Molecular Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

6.75

## PROFESSIONAL:

6.75

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Our goal is to understand how *Escherichia coli* coordinates overall characterized gene expression with particular attention given to the role played by the regulatory nucleotide, guanosine 3', 5'-bispyrophosphate (ppGpp). In order to explore the possibility that ppGpp functions as an intracellular equivalent of a hormone, we have characterized the relA gene, which encodes an enzyme that catalyzes synthesis of ppGpp on the ribosome in response to protein synthesis stalled for lack of aminoacylated tRNA and the spoT gene, which encodes a ppGpp 3' pyrophosphohydrolase and is the main route of cellular ppGpp degradation. In addition, we are characterizing the regulatory features of ppGpp effects at the transcriptional level. We have previously mutationally altered the spoT gene to varying degrees such that a range of ppGpp basal levels are obtained during steady state growth; these results indicate a likely regulatory role for ppGpp during normal growth in addition to dramatic changes in concentration during physiological stress. We have localized the spoT gene as an internal open reading frame within a multicistronic operon, about 1 Kb away from its promoter. Fusions of spoT operon transcripts suggest negative regulation by ppGpp. In collaboration with Dr. G. Glaser (Jerusalem), the relA gene has been localized in a monocistronic operon; fusions of its promoter to a lac reporter gene also indicate shares inhibition by ppGpp. The relA and the spoT gene sequences share considerable (40% identity) homology. Sequence verified null mutations in both the relA gene and the spoT gene have been prepared. The relA null allele shows a more severe phenotype than previously known relA mutations, that the relA gene is nonessential, and that an alternative pathway for ppGpp indeed exists. The spoT null allele behavior shows that previous spoT mutations were partially active and that deletion of the gene is lethal, probably because of the inhibitory effects of the ensuing high levels of ppGpp. The lethality of the spoT null allele in haploids can be reversed by suppressor mutations, which we suspect can lower ppGpp levels either by inhibiting alternative routes of ppGpp synthesis or stimulating alternative routes of degradation. Two such mutations have been isolated; one is located adjacent to the spoT locus and the other does not map near either the relA or spoT genes.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00068-16-LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Factors Influencing Genetics Transcription-Initiation and Termination

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R.J. Crouch Research Chemist LMG, NICHD

Others: L. Lempereur Visiting Fellow LMG, NICHD  
D. McKelvin Biologist LMG, NICHD  
J. Levin Research Biochemist LMG, NICHD

## COOPERATING UNITS (if any)

Dr. Breck Beyers, Dept. of Genetics, Univ. of Wash., Seattle, WA;  
Dr. M.L. Dirksen, Dermatology Branch, DCBD, NCI, NIH;  
Dr. Rick Bockrath, Indiana School of Medicine

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Molecular Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL

2.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

We have searched for changes in the growth of yeast and E. coli when ribonuclease H (RNase H) levels are below or above that found in normal cells. In E. coli, we have found that there are several different phenotypes including an increased sensitivity to UV irradiation. The results with E. coli suggest that DNA-RNA hybrid formation occurs under normal and stress situations and the activity of RNase H plays an important role in removing the hybrids to inhibit or permit the utilization of the RNA in the hybrids in DNA synthesis. In yeast, there is no strong phenotype yet detected when there is underproduction of RNase H.

Our studies on ribosomal RNA processing have centered on the yeast RRP-1 protein which is involved in the formation of mature 5.8 and 28S rRNAs. Nucleic acid sequence analysis suggest that RRP-1 is not a ribosomal protein.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00069-15-LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Aspects of the Replication of Mammalian Retroviruses

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith G. Levin Research Biochemist LMG, NICHD

Others: Ya-Xiong Feng Visiting Associate LMG, NICHD  
Robert Crouch Research Chemist LMG, NICHD  
Klara Post Biologist LMG, NICHD  
Michael Seddon Bio Aid LMG, NICHD  
Steve Joe SIS LMG, NICHD

## COOPERATING UNITS (if any)

NCI (Don Court, Dolf Hatfield, Brenda Gerwin); PRI-FCRF (Martin Zweig); BRI Basic Research Program, NCI-FCRF (Alan Rein)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Unit on Viral Gene Regulation (Developmental Biology Section)

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.8

## PROFESSIONAL

1.5

## OTHER

1.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the MuLV pol gene and on correlation of genetic structure with pol-associated enzymatic functions. Molecular clones containing MuLV reverse transcriptase sequences have been expressed in E. coli. The enzymatic activity of one of these clones, pRT250, has been characterized. The expressed protein differs from wild-type reverse transcriptase at the N-terminus, where there is a short leader peptide, and at the extreme C-terminus, where three amino acids have been changed and eleven others deleted. The pRT250 protein has been extensively purified and high levels of polymerase activity, with the characteristics of an MuLV reverse transcriptase, have been detected. Some RNase H activity is also observed; however, the polymerase to RNase H ratio is approximately 15-fold higher than that determined for a comparable preparation of the viral enzyme. These studies are consistent with the idea that DNA polymerase activity is localized to the N-terminal domain of reverse transcriptase and RNase H activity, to the C-terminal portion of the protein. Further experiments to explore this possibility are in progress. Work has continued on the reverse transcriptase-endonuclease complex in virions. More detailed analysis of the viral proteins indicates that intermolecular disulfide bonds are not involved. Recently, experiments designed to investigate the mechanism of suppression of the UAG termination codon separating the MuLV gag and pol coding regions have been initiated.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00071-15 LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Gene and Transgene Regulation in the Developing Mouse

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I. H. Westphal

Others: L. Crofford, Guest Researcher

M. Mangano, Guest Researcher

A. Dey, Visiting Fellow

R. Miskin, Guest Researcher

A. Griep, Staff Fellow

T. Nakamura, Visiting Fellow

J. Khillan, Visiting Fellow

S. Yu, Visiting Fellow

B. Krippl, Visiting Associate

E. Lee, Veterinarian

(All listed personnel affiliated with LMG/NICHD)

K. Mahon, Staff Fellow

## COOPERATING UNITS (if any)

NEI, NIH (J. Platigorsky, T. Kuwabara); NIAID, NIH (M.A. Martin);  
Harvard Medical School (C. Dawe); FCRF, NCI, NIH (F. Vande Woude)  
Max Planck Institute, Gottingen, West Germany.

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Mammalian Gene Regulation (formerly Section on Animal Viruses)

## INSTITUTE AND LOCATION

NICHD

## TOTAL MAN-YEARS

12.5

## PROFESSIONAL:

9.3

## OTHER:

3.2

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Our laboratory investigates mechanisms of gene control in the mouse. The first part of the project concerns an endogenous gene containing a homeo box sequence. This gene is likely to play a role in morphogenesis of the embryo. Transcripts localize to specific regions of the developing central nervous system and to segmented structures including the spinal ganglia and sclerotomes. All other parts of the project utilize the transgenic technology which allows us to measure the regulation of a given gene construct under real in vivo conditions at any stage of development of the mammalian organism. Transgenes were introduced in the mouse germline by DNA microinjection in the one-cell embryo. Two of our studies deal with the action of an oncogene and a protooncogene, respectively, in the ocular lens. In the presence of SV40 T antigen, transformation occurs during primary lens fiber differentiation in the embryonic eye. A vascularized, invasive cancer evolves at later stages of development. Expression of the *c-mos* protooncogene, on the other hand, interferes with specific stages of secondary lens fiber differentiation but does not result in hyperplasia or neoplasia of lens tissue. In another part of the project, we have utilized genetic elements of polyoma virus to effect overreplication, episomal propagation and increased expression of transgenes. The latest study deals with a mouse model for investigating activation of the human immunodeficiency virus (HIV). Mitogens and viruses suspected as co-factors in AIDS disease have been demonstrated to activate HIV long terminal repeat sequences in the transgenic mouse.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01001-05 LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Organization and Expression in *Drosophila*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. Dawid	Head	LMG, NICHD
Others:	S. Haynes	Senior Staff Fellow	LMG, NICHD
	B. Mozer	Biologist	LMG, NICHD
	N. Bhattia-Dey	Guest Researcher	LMG, NICHD
	D.-H. Huang	Guest Researcher	LMG, NICHD
	D. Henderson	SIS	LMG, NICHD

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Developmental Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.6

## PROFESSIONAL:

2.4

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular-genetic studies have continued on the maternal effect homeotic gene *fs(1)h* of *Drosophila*. Overlapping cDNAs corresponding to the major ovarian transcripts of 7.6 and 5.9 kb have been almost completely sequenced. The 5.9 kb mRNA sequence predicts a protein of a size of approximately 110 kd. The predicted protein is very rich in glycine, alanine and serine, some of which occur as clusters. The protein contains 6 potential asparagine-linked glycosylation sites and two potential transmembrane domains. Antisera have been prepared against two fusion proteins that contain portions of the predicted *fs(1)h* product. These sera detect a 70 kd protein in *Drosophila* extracts, suggesting the possibility that the transcript has undergone processing. The *fs(1)h* protein is expressed ubiquitously in the embryo; its distribution in the cell is compatible with properties of a membrane protein.

The *trithorax* (*trx*) gene is a major regulatory developmental locus in *Drosophila* that interacts with *fs(1)h* and affects the expression of other homeotic loci in the bithorax and Antennapedia complexes. The *trx* gene has been cloned by isolating the region interrupted by P elements in two independently derived dysgenic mutant alleles. A small ubiquitously expressed RNA and a large RNA specific for larvae and pupae have been detected by probing with *trx* sequences. cDNAs possibly representing these transcripts have been isolated and are being sequenced.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01002-05 LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Gene Expression During Embryonic Development of *Xenopus laevis*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: I.B. Dawid, Head (All personnel listed below are associated with LMG/NICHD)

Others: T. Sargent, Senior Staff Fellow D. Henderson, SIS  
S. Miyatani, Visiting Fellow M. Rebbert, Chemist  
M. Jamrich, Visiting Scientist S. Sato, Senior Staff Fellow  
E. Jonas, Visiting Associate M. Jacobson, IPA  
A. Cheng, Biologist H. Grunz, Courtesy Contract  
G. Michaels, Staff Fellow K. Richter, Visiting Fellow  
S. LaFlamme, Guest Researcher

## COOPERATING UNITS (if any)

L. Charnas and H. Gainer, HGB, NICHD, & LNN, NICHD, & LNC, NINCDS  
R. Friesel, T. Maciag and J. Winkles, Red Cross Laboratories

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Developmental Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

9.4

## PROFESSIONAL:

7.9

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This work aims to elucidate molecular events during early amphibian embryogenesis. To this end molecular markers specific for early differentiation events are being isolated and studied. The DG42 gene which is expressed only during gastrula and neurula stages has been studied. An antibody produced against the predicted DG42 protein allowed localization of the protein in the inner but not outer layer of the ectoderm and in the endoderm. Ectodermal expression is terminated at the time of neural induction during gastrulation. Constructs of keratin and DG42 genes have been injected into the *Xenopus* embryo; preliminary evidence has been obtained for temporally and spatially regulated expression of these introduced genes. A new marker for notochord differentiation has been isolated. This gene is a member of the intermediate filament superfamily; it is expressed at low levels in all tissues tested, but is very much enhanced in the notochord. Induction of mesoderm has been studied with the aid of a factor secreted by a *Xenopus* cell line named XTC. This crude factor induces morphological differentiation towards mesodermal derivatives in cells that otherwise would become ectoderm. In addition, the expression of the mesodermal marker genes  $\alpha$ -actin and notochord-specific filament is induced, while the expression of keratin genes (ectoderm specific) is repressed by XTC factor. Mammalian fibroblast growth factor (FGF) and its acidic form, also called endothelial cell growth factor (ECGF), mimick some of the inductive effects of XTC factor. The possible relationship between these factors is being studied.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01004-04 LMG

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Regulation of Amino Acid Biosynthetic Genes in *Saccharomyces cerevisiae*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Alan G. Hinnebusch	Senior Staff Fellow	LMG, NICHD
Others:	Paul Miller	NRC Fellow	LMG, NICHD
	Norma Williams	Guest Researcher	LMG, NICHD
	Ernest Hannig	Staff Fellow	LMG, NICHD
	Peter Muller	Visiting Fellow	LMG, NICHD
	Gary Fabian	Staff Fellow	LMG, NICHD
	Chris Paddon	Visiting Associate	LMG, NICHD
	Belinda Brown	Biologist	LMG, NICHD
	Deborah Crouch	Guest Researcher	LMG, NICHD

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section of Developmental Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6.42

## PROFESSIONAL:

5.60

## OTHER:

0.83

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The GCN4 protein activates transcription of multiple amino acid biosynthetic genes in yeast. GCN4 expression is regulated at the translational level by AUG codons in the 5' leader of its mRNA. Either the third or the fourth AUG codon (from the 5' end) is needed for efficient repression in non-starvation conditions; the first AUG codon is required for derepression in starvation conditions. Positive (GCN) and negative (GCD) trans-acting factors modulate the effects of the upstream AUG codons. We have made the following advances in our understanding of the molecular functions of these cis and trans-acting control elements: (1) It was shown that a 240 base leader segment containing all four AUG codons confers translational control typical of GCN4 upon a heterologous yeast transcript. Deletions of sequences between short open-reading-frames (ORFs) 1 and 4 destroyed regulation, showing a requirement for sequences in this region for translational control. The termination codon of ORF 4 was shown to be dispensable; however, removal of the termination codon of ORF 1 impaired regulation. (2) gcd mutations were shown to produce a general decrease in the rate of misreading at stop codons in vivo (antisuppression); mutations in two omnipotent suppressors, sup35 and sup46, have a Gcn<sup>-</sup> phenotype. (3) The GCD12 gene has been cloned, its functional unit mapped by deletion analysis, and localized near the centromere on chromosome VII. (4) Expression of GCN3 was shown to be unaffected by amino acid availability, suggesting that increased GCN3 regulatory function in starvation conditions results from activation of the gene product by protein modification.



LABORATORY OF NEUROCHEMISTRY AND NEUROIMMUNOLOGY

Z01 HD 00056-12    Biosynthesis, Processing & Secretion of Neuropeptides  
                            & Pituitary Peptide Hormones  
                            Yoke Peng Loh, Ph.D.

Z01 HD 00705-06    Functional Organization of the Nerve Terminal  
                            James Russell, Ph.D.



NICHD ANNUAL REPORT  
October 1, 1986 to September 30, 1987

Laboratory of Neurochemistry and Neuroimmunology

This laboratory is concerned with the development, functional organization and interactions between two major integrative systems in the body - the central nervous system and the endocrine system. The approach of the laboratory is cell biological in nature, and hence utilizes a wide variety of techniques and concepts from a number of disciplines, e.g., physiology, biochemistry, morphology, immunology, and molecular biology. In particular, we study various secretory peptides, intracellular membrane systems, and cytoskeletal proteins which are found in these organ systems and which are essential to their functions (i.e., peptide biosynthesis and regulation, neuronal morphology and function, etc). A special emphasis is placed on the study of the cellular development of these organ systems.

The activities of the laboratory were divided into two sections and one unit. As of March, 1987, the Section of Functional Neurochemistry headed by Dr. Harold Gainer was transferred to NINCDS. In April, 1987, the Unit on Neuronal Secretory Systems was transferred to LDN, NICHD.

I. Section on Cellular Neurobiology

The research goal of this Section is to study brain and pituitary peptides which are involved in intercellular neurocommunication and neural development.

The emphasis has been on the ACTH/endorphin/ $\alpha$ -MSH family of peptides. Endorphin is an opiate peptide that is found in brain, pituitary and placenta.  $\alpha$ -MSH is present in brain and pituitary, but in humans it is present in the pituitary only during pregnancy and in the fetus. This peptide has been implicated to play a role in osmoregulation, fetal growth and morphogenesis. ACTH is a pituitary peptide which stimulates steroidogenesis and is a mediator of stress. All these peptides have been shown to have various central nervous system effects and are thought to act as neurotransmitters and neuromodulators. The major focus has been to continue to study the enzymology and regulation of biosynthesis, packaging and secretion of this family of peptides, their anatomical localization and their role in nervous system function and development.

The ACTH,  $\alpha$ -MSH and endorphin peptides are synthesized in the intermediate lobe of the pituitary from a common, glycoprotein prohormone (pro-opiomelanocortin, POMC) of about 32,000 daltons in size. We have assayed for several enzymes involved in the processing of this prohormone. These include a carboxypeptidase B-like enzyme, an



aminopeptidase B-like enzyme and a paired basic residue-specific prohormone converting enzyme (PCE). This latter enzyme has been purified to apparent homogeneity from secretory vesicles of the bovine pituitary intermediate lobe and neural lobe. PCE from both lobes appear to have very similar characteristics and are likely to be the same enzyme. PCE is a glycoprotein, has a molecular weight of ~70,000 daltons and cleaves several precursors (POMC, pro-vasopressin, pro-insulin and pro-enkephalin) at paired basic residues to yield products seen in the tissues that synthesize these prohormones or neuropeptide precursors. Inhibitor studies have shown that PCE is inhibited by two aspartyl protease inhibitors, pepstatin A and diazoacetyl-norleucine methyl ester, but not by thiol or serine protease inhibitors. Further evidence that PCE is a physiologically significant enzyme in prohormone processing comes from Dr. Castro's recent studies showing that the enzyme was secreted from dissociated bovine intermediate lobe cells together with the hormone,  $\alpha$ -MSH, in a coordinately regulated manner. Finally, we have demonstrated that POMC processing was inhibited by pepstatin A in intact mouse neurointermediate lobes. This latter result shows that PCE has fulfilled the most stringent criteria for the identification of a physiologically relevant prohormone processing enzyme as set forth by Docherty and Steiner (Ann. Rev. Physiol. 1982) i.e., an inhibitor of the putative enzyme must interfere with the precursor processing in the intact cells. Dr. N. Birch is in the process of cloning this enzyme. He has a few promising clones derived from screening an Okayama Berg library from AtT-20 cells. PCE is the first enzyme demonstrated to process intact prohormones and is of potential commercial value for the production of peptide hormones and neuropeptides when used for the limited cleavage of precursors synthesized by bacteria which has been transfected with a vector carrying the prohormone cDNA sequence. A patent for this enzyme is pending.

Dr. Nigel Birch has been working on an aminopeptidase B-like enzyme from pituitary secretory vesicles. He has shown that the enzyme cleaves an Arg at least 6-fold faster than a Lys at the N-terminal of a peptide. This result is exciting since it can explain the accumulation of high levels of Lys- $\gamma$ -MSH in the intermediate pituitary, but no other Arg extended POMC derived peptides, except when the Arg is followed by a Pro, as in CLIP. In this case the amino-peptidase B-like enzyme will not cleave the Arg. He has shown that this enzyme is secreted from bovine intermediate lobe cells with  $\alpha$ -MSH in a regulated manner.

Katrin Andreasson has used a transient expression system employing a vector with a bacterial T7 promoter and a recombinant vaccinia virus carrying the T7 polymerase gene to express prohormones (POMC and pro-vasopressin) in CV1 cells for use as substrates for the processing enzymes. This system was also used to express constructs of truncated POMC/CAT fusion genes in a model secretory cell (AtT-20 cell) to study the molecular domains necessary for the routing of the prohormone into secretory vesicles. Using an immunocytochemical assay to detect CAT at the E.M. level, K. Andreasson, in

collaboration with Dr. Vaidya, showed for the first time that the molecular structure essential for the correct transport of POMC from the rough endoplasmic reticulum to the secretory vesicle lies within the first 25 amino acids of the prohormone. Efforts will be made to determine if the signal directing other prohormones into the regulated secretory pathway of cells also lies within the NH<sub>2</sub> terminus of the molecule. This study opens the possibility of designing appropriate ligands to search for intracellular receptors involved in directing proteins to the regulated secretory pathway, analogous to the mannose -6- phosphate receptor for sorting lysosomal proteins.

The regulation of POMC synthesis has been studied in the frog and mouse pituitary. B. Myers, in collaboration with Dr. T. Zoeller, using a synthetic 48 mer oligonucleotide probe to POMC and quantitative *in situ* hybridization, showed that POMC mRNA levels in the intermediate lobe increased 4-fold, as did POMC synthesis during black background adaptation of the frogs. Dr. Stela Elkabes measured POMC mRNA levels and POMC synthesis in the pituitary of mice that were subjected to hypertonic stress by replacing their drinking water with 2% saline. Salt-loading for two days resulted in an increase in POMC mRNA levels in the anterior pituitary and an increase of plasma ACTH. POMC synthesis and processing did not change in this tissue. In contrast, POMC mRNA levels and synthesis decreased in the intermediate lobe as did plasma  $\alpha$ -MSH. These results show that POMC synthesis can be independently regulated at the transcriptional and translational level. CRF and AVP neurons, which may regulate the change in POMC synthesis in the anterior pituitary during salt-loading, were studied by Dr. Elkabes in collaboration with Dr. S. Young using the quantitative *in situ* hybridization techniques to measure mRNA levels. Changes in AVP and CRF mRNA levels in these neurons generally reflect the secretory activity. The studies revealed that AVP but not CRF mRNA in the hypothalamic neurons were increased after 2 days salt-loading. Furthermore, immunoreactive CRF in the median eminence (the release site) was unaltered, but plasma AVP increased. These results suggest that AVP may play a key role in potentiating POMC synthesis and ACTH release in the anterior pituitary during hypertonic stress. This is similar to some other stresses, e.g. when psychiatric patients are given electroshock treatment and during hypoglycemic or hemorrhagic stress, no change in portal or peripheral blood CRF is observed, but plasma AVP is increased significantly.

The role of AVP in attenuating CRF action was studied by Dr. Maria Castro. She showed that treatment of mouse anterior lobe cells with a constant submaximal dose of CRF (0.1 mM) and increasing doses of AVP (0.01 - 10  $\mu$ M) gave rise to a linear increase in ACTH secretion. However, treatment with a constant level (0.1 mM) of AVP and increasing concentrations of CRF rapidly resulted in a leveling off of ACTH secretion at 1.0 nM CRF concentration. These results indicate for the first time that fine attenuation of ACTH secretion can be best achieved with a constant low level of CRF and differing levels of AVP, rather than by maximally increasing CRF alone. Dr. Castro

has also shown that with prolonged CRF treatment (>6h) at 1 nM concentration, there was a progressive decrease per unit time of ACTH secretion from mouse anterior pituitary cells. In the light of Dr. Castro's results, CRF levels may be increased only transiently in vivo, perhaps in response to acute stress, but such increases may not be sustained for extensive periods during stress.

Recently, Dr. Stela Elkabes has initiated a new project on the expression of POMC during development of the CNS in the mouse. Immunocytochemical techniques were used to determine the earliest embryonic stage at which POMC is expressed in the brain. POMC and/or POMC related peptides have been detected in neurons and neural projections at embryonic day 10-1/2, the earliest stage studied. POMC-containing cells were not detected in the Rathke's pouch (precursor cells to the pituitary) until embryonic day 13-1/2. In a comparative study with LHRH and AVP carried out by Drs. Wray and Neiburg (NINCDS), these peptidergic systems were seen at a later embryonic stage in the brain than POMC. This is very interesting since it would suggest that the POMC related peptides may play a role in very early neurogenesis. We now plan to determine the POMC processing pattern in the brain in early embryonic development and test the appropriate POMC related peptides present on neuronal cultures for morphogenic and mitogenic activity.

#### Unit on Neuronal Secretory Systems

Secretion of neurotransmitters and other biologically active substances from nerve terminals forms the fundamental mechanism from the time of development to higher order central nervous system functions in the mature adult. Transduction of information content in the action potential train, to modulation of transmitter release by local influences at the nerve terminal via receptors is thought to be the basis of most CNS functions. Thus, the nerve terminal is a highly specialized region of a neuron, separated from the neuronal soma by an axon, whose function is to release neurotransmitter quanta and to regulate the number of quanta secreted. Modulation of the quantity of the transmitter released at the terminal may form the basis for all central nervous system functions, including integration of information, and long term information storage, and retrieval. Because of the complexity (cellular heterogeneity, and their complex organization), and extremely small size, basic understanding of the molecular mechanisms of nerve terminal function in the central nervous system is lacking. The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. The nerve terminals of the neurons of the hypothalamo-neurohypophysial system are discretely localized in the neurohypophysis, where they are accessible to experimental manipulations both in vivo and in vitro. These nerve terminals could be isolated from the neurohypophyses without contamination by the post-synaptic membrane, unlike nerve terminals from other regions in the central nervous system. Studies on the



elucidation of the functional organization of the nerve terminal forms the central theme of the Unit on Neuronal Secretory Systems. The current focus of the Unit is on the investigation of the importance of ionic channels and receptors on the initiation and modulation of secretion at the nerve terminal. Dr. Carolyn Bondy's experiments have shown that a type of  $K^+$  channel may play a central role in modulation of secretion at the terminal caused by frequency information in the action potential train. The identification of a specific peptide toxin against a  $K^+$  channel provides an unique opportunity to characterize this channel. This will be the first direct biochemical identification of a  $K^+$  channel and hopefully will allow us to obtain a molecular description of neuronal excitability.

The neurosecretosome preparation has been maintained in culture for long periods of time. This basic preparation for the first time allows for experiments on identification of ionic channels on nerve terminals using state-of-the-art biophysical techniques so that the channels, and their modulation by neuropeptide receptors on the nerve terminals could be investigated. In collaboration with Dr. Elis Stanley, patch-clamp techniques will be used to characterize both the  $Ca^{++}$  and  $K^+$  channel types present on the nerve terminals. To date, no such information is available in vertebrate nerve terminals. This neurosecretosome preparation also provides an ideal model to study intracellular reactions involved in triggering and regulation of neurosecretion. The use of toxins that block secretion is expected to provide a means of identifying cellular substrates important in the exocytosis machinery. The high resolution video imaging microscope adds a new dimension in the investigation of the nerve terminal organization. The long standing questions on the kinetics of  $Ca^{++}$  concentration increase in the terminal and its homeostasis will be initially studied.

Dr. Bondy has completed her studies on stimulus frequency-dependent, and opiate receptor coupled modulation of secretion at the neurohypophysial nerve terminals. Dr. James Y. Garbern has succeeded in obtaining a partially purified fraction of high affinity calcium binding protein unique to nerve terminals. Dr. Holly I. Trenchard joined the Unit recently as an IRTA Fellow and is involved in the identification of the dendrotoxin binding  $K^+$  channel in the posterior pituitary nerve terminals.

The Unit on Neuronal Secretory Systems has been a part of the Laboratory of Neurochemistry and Neuroimmunology. In April 1987, this Unit was transferred to the Laboratory of Developmental Neurobiology.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00056-12 LNN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis, processing &amp; secretion of neuropeptides &amp; pituitary peptide hormones

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Y. P. Loh	Head	LNN, NICHD
Others:	Nigel Birch	Visiting Fellow	LNN, NICHD
	Stela Elkabes	Visiting Fellow	LNN, NICHD
	Maria Castro	Visiting Fellow	LNN, NICHD
	Fan-Jie Zeng	Visiting Associate	LNN, NICHD
	Winnie Tam	Microbiologist	LNN, NICHD
	Brenda Myers	Junior Fellow	LNN, NICHD
	Katrin Andreasson	Hughes Fellow	LNN, NICHD

## COOPERATING UNITS (if any)

Lab. of Cell Biology, NIMH (T. Zoeller, M. Brownstein, S. Young III & H. Okayama);  
 Lab. of Viral Diseases, NIAID (B. Moss & T. Fuerst); Lab. of Neurochemistry, NINCOS  
 (H. Gainer, S. Wray & A. Nieburg); Unif. Serv. Univ. Health Sci. (U. Patel-Vaidya)

## LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

## SECTION

Section on Cellular Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS

6.75

## PROFESSIONAL:

4.25

## OTHER:

2.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pituitary secretory vesicle enzymes involved in the processing of pro-opiomelanocortin (POMC, pro-ACTH/endorphin) and pro-vasopressin were studied. A paired, basic residue specific prohormone converting enzyme (PCE) previously purified and characterized as an aspartyl protease was shown to be secreted from bovine intermediate lobe together with  $\alpha$ -MSH, in a co-ordinately regulated manner. Pepstatin A, an inhibitor of PCE, blocked processing of POMC in the mouse intermediate lobe further supporting a physiological role of the enzyme in vivo. The POMC and pro-vasopressin genes have been transfected into CV1 cells (green monkey kidney cell line) using a vaccinia virus transient expression system. The transfected cells secrete these prohormones in the intact form which can be recovered for use as substrate for PCE. An aminopeptidase B-like enzyme which removes basic residues from peptides cleaved by PCE was also shown to be co-secreted with  $\alpha$ -MSH from bovine intermediate lobe cells in a regulated manner. The effect of salt-loading on POMC biosynthesis in the pituitary was studied. POMC mRNA levels, POMC synthesis and plasma  $\alpha$ -MSH levels decreased in the intermediate pituitary after 2 days of salt-loading and returned to normal after 9 days. In contrast, anterior pituitary POMC mRNA levels and plasma ACTH increased after 2 days of salt-loading and returned to normal by 4 days. In situ hybridization analyses of CRF and vasopressin (AVP) mRNA levels in hypothalamic neurons during salt loading showed no change in CRF but an increase in AVP mRNA levels. Studies on the interaction of AVP and CRF show that AVP is highly effective in attenuating CRF action at low doses of CRF.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00705-06 LNN

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional organization of the nerve terminal

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. Russell Head LNN, NICHD

Others: C. A. Bondy Medical Staff Fellow LNN, NICHD  
J. Y. Garbern Medical Staff Fellow  
H. I. Trenchard IRTA Fellow LDN, NICHD

## COOPERATING UNITS (if any)

Laboratory of Biophysics, NINCDS (G. Ehrenstein, S. Ellis)

## LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

## SECTION

Unit on Neuronal Secretory Systems

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.5

## PROFESSIONAL:

3.0

## OTHER:

.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Our research program is directed towards studying the biochemistry and physiology of the nerve terminal. The neurohypophysial nerve terminals are used as the model system, and secretion of vasopressin and oxytocin from isolated intact posterior pituitaries and isolated neurosecretosomes, and ionic channels on the neurosecretory vesicle membrane are studied. The secretion of peptides from intact neural lobes was found to be facilitated by increasing the stimulus frequency. Experiments using K<sup>+</sup> channel blockers suggested that action potential widening caused by inactivation of transient K<sup>+</sup> channels may form the molecular basis of this frequency-dependent facilitation. A neurotoxin from Dendroica Angusticeps which blocks transient K<sup>+</sup> channels was found to enhance hormone secretion under low frequency stimulation, suggesting that these channels may be involved in the innate facilitation. Secretion from oxytocin terminals was found to be inhibited by the opiate  $\kappa$  receptor agonist, dynorphin, released from vasopressin terminals. The nerve endings (neurosecretosomes) were shown to contain  $\kappa$  type opiate receptors. The preparation of neurosecretosomes has been maintained under tissue culture conditions for over a week in order to study the kinetics of neuropeptide secretion in vitro. Methods have been developed to study hormone secretion and binding of ion channel blockers and receptor probes in these nerve endings. This preparation is also suitable for high resolution fluorescence and differential interference contrast microscopy and analysis of ionic channels. Patch clamp studies on intermediate lobe cells revealed the presence of three different types of calcium channels. A method was developed for classification of these three types of channels based on their inactivation kinetics. Bilayer incorporation studies using isolated neurosecretory vesicles have led to the identification of a calcium-activated cation channel with large conductance (>400 PS) and an anion channel on these membranes. These channels may be important in the mechanism of exocytotic secretion. A novel high affinity calcium binding protein was identified in nerve endings of the posterior pituitary and partially purified.





LABORATORY OF THEORETICAL AND PHYSICAL BIOLOGY

- Z01 HD 00040-12 Statistical and Mathematical Studies of Molecular Interactions  
Peter J. Munson
- Z01 HD 00165-12 Isolation and Characterization of Macromolecular and Cellular Particles  
Andreas Chrambach, Ph.D.
- Z01 HD 00171-11 Electrophoretic Methodology  
Andreas Chrambach, Ph.D.
- Z01 HD 00189-06 Computer Programs for Analysis of Laboratory and Clinical Data  
David Rodbard, M.D.
- Z01 HD 01400-05 Clinical Applications of Stable Isotopes  
Alfred L. Yergey, Ph.D.
- Z01 HD 01401-05 Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry  
Alfred L. Yergey, Ph.D.
- Z01 HD 01404-04 Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues  
David Rodbard, M.D.
- Z01 HD 01405-03 Computer Programs to Aid Intensive Insulin Therapy for Type-1 Diabetes Mellitus  
David Rodbard, M.D.

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NICHD Annual Report  
October 1, 1986 to September 30, 1987

Laboratory of Theoretical and Physical Biology

This multidisciplinary laboratory is involved in development of new and novel applications of mathematical modelling, statistical analysis, computational methods, ligand binding studies, thermospray liquid chromatography/mass spectrometry, gel electrophoresis, isoelectric focusing and related physical chemical methods to significant areas in biology and medicine.

Several of our studies are directly relevant to reproductive endocrinology, e.g. development of sensitive, specific, computerized statistical methods for automated objective measurement of episodic secretion of LH, FSH, and other hormones, and characterization of receptors for opioids, vasopressin, and oxytocin in brain and both male and female genital tracts. Further, the laboratory is engaged in clinical investigations of inborn errors of metabolism, (e.g. glycogen storage disease type I), studies of calcium and mineral metabolism under a wide variety of conditions (infants, neonates, normal growth, puberty, pregnancy, lactation, aging, osteoporosis) and in various disease states (vitamin D resistant rickets, fibrodysplasia ossificans progressiva).

We also study a variety of endocrine conditions, e.g. cortisol production rate in patients with Cushing's syndrome, Addison's disease, and hypercortisolemia of diabetes mellitus. The techniques developed for study of cortisol will be adapted to the study of testosterone metabolism in polycystic ovarian disease and idiopathic hirsutism, and to the study of vitamin D metabolism in normal subjects and in a variety of pathophysiological states.

We have developed improved methods for the characterization of peptides (e.g. growth factors) and proteins, using thermospray liquid chromatography/mass spectrometry employing quadrupole technology and time-of-flight detection, and using gel electrophoresis and isoelectric focusing.

Section on Theoretical Biology:

The section directed by D. Rodbard is engaged in combined theoretical-experimental studies of receptors for drugs, hormones and neurotransmitters with special reference to endocrinology and reproductive biology. This group has placed special emphasis on receptors for vasopressin and oxytocin, opioid peptides, and the drug of abuse, phencyclidine.

1. In the previous year, this group demonstrated the presence of high concentrations of receptors for vasopressin and oxytocin throughout the male genital tract, including testis, Leydig cell, vas deferens, epididymis, and seminal vesicle. These receptor systems are likely to be involved in modulating the contractility of these structures and various metabolic processes, e.g., fructose metabolism in the seminal vesicle. The use of mathematical modelling made it possible to resolve the two types of receptors, which would have been missed by prior conventional methods of analysis. This raised the possibility that vasopressin and oxytocin receptors could coexist in the classical target tissue (uterus) previously thought to contain only oxytocin receptors. This led to the demonstration of both vasopressin and oxytocin receptors in uterus, vagina, and

oviduct of the rabbit. These receptors respond to hormonal modulation: both increase in a dose dependent fashion with estrogen, an effect blocked by progesterone. During pregnancy receptor levels are similar to those expected in the presence of elevated levels of estrogen and progesterone. At the time of delivery, there is a dramatic increase in levels of oxytocin receptors, but not of the vasopressin receptors and there is a decrease in vasopressin receptor levels in vagina. Both types of receptors appear to be coupled to muscular contractility. The exact anatomical location of these receptors, and their mechanisms of signal transduction, remain to be explored.

2. The drug, (+)-N-allyl-normetazcine (SKF 10,047) induces hallucinations and other psychotomimetic effects. At first it was believed that its effects are mediated by opioid receptors. However, recent evidence from several laboratories suggested that its effects were mediated via a distinct, non-opioid receptor, the "sigma" receptor. There followed considerable controversy whether the receptor for SKF 10,047 was the same as for phencyclidine (PCP), a drug of abuse ("angel dust"). We have used an approach, previously developed in this laboratory for analysis of opioid and vasopressin-oxytocin receptors, to help resolve this controversy. Our results unequivocally demonstrate that the SKF and PCP sites in rat brain are distinct. Our approach should also be useful in characterizing these receptors in other tissues, e.g., anterior pituitary. Improvement of the radio-receptor assay may assist in the development of new drugs (agonists and antagonists) with improved specificity for the desired site.

3. Using mathematical theory, we have developed methods to optimize experimental design for ligand binding studies. This is especially important when the biological tissue is precious or in limited supply (e.g., steroid receptors in specimens of human breast carcinoma, neuropeptide or other receptors in hypothalamus or pituitary). A practical computer program has been developed, to permit selection of optimal concentrations of drug (ligand) to obtain the most precise estimates of binding capacity and affinity. The program can handle both simple cases (1 ligand, 1 receptor), or rather complicated cases such as the opioid, vasopressin-oxytocin, or SKF-PCP systems involving many ligands and many classes of binding sites. The program is especially important in the more complex cases, which are too complicated for analysis by "intuition" or subjective reasoning. General guidelines are also being developed, for experimentalists who do not have appropriate access to the computer program.

4. Most scientific data analysis is still based on subjective visual analysis of graphs (dose response curves, response versus time curves, etc.). Usually the experimentalist does not have a suitable mathematical model or formula available. We have developed a "self-modelling" approach, to combine the advantages of the empirical and the mathematical modelling approaches to data analysis. Simultaneous analysis of multiple curves (from various subjects, experiments, or treatments) allows one to identify the common curve-shape or template. Then, our computer program "FLEXIFIT" allows one to test hypotheses, obtain standard errors of parameters, and obtain the best possible scaling factors to permit pooling of data from multiple curves. This method and program should make objective, computerized, statistical analysis accessible to a wide range of biomedical scientists, for use on a wide range of problems not previously amenable to statistical or modelling analyses.

## Section on Metabolism and Mass Spectrometry:

This section led by A. Yergey has made important advances in the application of mass spectrometry to the determination of compounds and elements of biological and clinical interest. Stable isotopes, which can be most effectively measured only by mass spectrometry, are used as tracers and measurement standards.

The section has developed several important methods for analysis of carbohydrates, steroids and their metabolites using thermospray liquid chromatography/mass spectrometry (ThLC/MS). These methods provide molecular weight information on intact labile molecules without resorting to the chemical derivitization commonly required for GC/MS measurements. The methods for measurement of deuterium and  $^{13}\text{C}$  labelled tracers of steroids and carbohydrates at low levels of enrichment in human plasma (typically 1-3% of circulating levels of the native materials) have been applied to the determination of cortisol and glucose production rates (PR) in humans, and have led to important new clinically significant findings in glycogen storage disease and glucocorticoid metabolism. Using a tridutero cortisol tracer, Yergey's group (in collaboration with the Developmental Endocrinology Branch, NICHD) has demonstrated that the normal cortisol production rate in man is only 50-60% of previously accepted estimates. This clinically and diagnostically important result has been validated by measurements in adrenalectomized subjects by showing that the observed values of PR agreed with those expected from the ratio of natural and labelled cortisol infused. Further validation of the method is expected from the identification of the urinary metabolites of cortisol containing the deuterium label. Determination of these metabolites as the intact conjugates glucuronides, etc., is possible only through the use of ThLC/MS. In addition, this approach has recently been used to show elevated cortisol production rates in patients with Cushing's syndrome under therapy with o,p'-DDD.

A glucose tracer containing a  $^{13}\text{C}$  at every site has been used in studies of hepatic glucose production in Type-I glycogen storage disease (in collaboration with the Human Genetics Branch, NICHD) using the ThLC/MS technique. These studies reveal the presence of hepatic glucose production even in patients with no detectable glucose-6-phosphatase activity in liver biopsy specimens. Further, serum lactate levels are closely correlated with hepatic glucose production. These findings suggest a new set of therapeutic goals in management of these patients, i.e., blood glucose levels should be adjusted, by dietary means, to suppress serum lactate levels thereby reducing hypertriglyceridemia, hyperuricemia and other metabolic complications of this disorder.

Several preliminary studies show promise for future applications of ThLC/MS. The ability to determine vitamin D and its 1-hydroxy metabolite as intact, underivatized molecules in human plasma, has been demonstrated, and will be used in conjunction with tridutero labelled tracers in studies of vitamin D metabolism. Mass spectra of the other metabolites, including physiologically active 1,25 dihydroxy-vitamin D have been obtained as intact molecules, but assay sensitivities are not adequate for tracer studies. These ThLC/MS spectra of vitamin D and its metabolite are the first reported that contain molecular ions of these materials eluting from an LC. This instrument will be particularly important in continuing studies of autocrine growth factors and other small peptides with molecular weights up to 1500 daltons.



Another major focus of the activities of this section is the elucidation of whole body calcium metabolism through the use of stable isotopic tracers. Two different highly enriched, but naturally occurring, minor isotopes of calcium are given as tracers to a subject simultaneously. One is given orally, the other intravenously. Perturbation of the natural calcium isotope ratios by these tracers in blood, urine and feces are measured by thermal ionization mass spectrometry as a function of time after administration and are used, in conjunction with a mathematical model of a calcium whole body calcium distribution, to quantify fractional absorption and bone turnover dynamics in humans. Yergey's group has used this approach to develop a new, 24 hour test for fractional absorption of calcium from dietary sources. This procedure obviates the need for using radioactive tracers or a 15-day balance study. This benign method has been used to measure fractional absorptions in six premature infants: values ranged from 30-70%. These same measurements were used to show that the time rate of absorption in these infants was about twice that of normal children and adults. Absorption measurements in normal adult women have shown no age dependence in total absorption, but exhibit evidence for an age dependence of the time rate of absorption.

Studies of whole body calcium distribution conducted over an extended six-week period in several normal lactating women and in an osteoporotic woman have shown the existence of an anticipated, but hitherto unidentified slow turn-over component of calcium dynamics. This component of the distribution is almost certainly associated with a deeper bone turnover than has been previously identified directly by any analytical technique. This new approach promises to be useful in study of whole-body calcium turnover in osteoporosis hyperparathyroidism, familial hypercaluric hypercalcemia, and vitamin D resistant rickets.

#### Section on Macromolecular Analysis:

The section directed by A. Chrambach has conducted studies aiming at an accurate assessment of the surface net charge of macromolecules and of virus-sized particles by simple, gel electrophoretic means. Surface net charge is biologically important since many interactions of macromolecules and viruses with cells are of ionic nature, and thus many biological activities of such species are related to their charge. These workers found during the past year, that the common point of intersection of linear "Ferguson plots" of log (mobility) versus gel concentration for variously crosslinked gels provides a measure of protein net charge, and not, as held for the past two decades, the extrapolated intercepts on the mobility axis of the plot. The intersection point coincides with the transition from a polyacrylamide gel to a fluid polymer at about 2% gel concentration.

Independent evidence that a linear extrapolation of "Ferguson plots" does not yield accurate free mobility values, taking advantage of the possibility to measure mobilities of virus-sized particles in the liquid polymer showed that in the "liquid" concentration range the plot was convex, and that particle net charge could be accurately assessed by non-linear extrapolation of the convex plot to zero gel concentration. Values agree with those derived from agarose gel electrophoresis, using the same approach.

Chrambach's section took still another approach towards an assessment of particle net charge, viz. two-dimensional electrofocusing-gel electrophoresis

which yielded, for the first time, experimental titration curves of viruses. These are useful by providing not only the isoelectric point of the particle, but at the same time delineating pH regions of maximal mobility as well as of denaturation; moreover, the sigmoidal transitions in the titration curves may potentially be used to identify the titrated functional group on the particle. Titration curves, like "Ferguson plots", can serve as "fingerprints" for a subcellular particle. Computer programs were developed to normalize the irregular experimental pH profiles on the two-dimensional gels, allowing for the comparison of titration curves between experiments.

The development of a practical procedure for obtaining stationary two-dimensional electrofocusing--SDS-PAGE patterns was continued by solving the problem of apparent non-migration of some proteins in excess of a size of 200 kD. The retention of these proteins was shown to be due to an excessive Immobililine concentration and an insufficient carrier ampholyte concentration in the gel. Thus, the remedy consisted of optimizing the ratio between these two concentrations. As a consequence of that development, a procedure of mapping cellular proteins is available for the first time since O'Farrell innovated the method 12 years ago which can provide constant multicomponent patterns, which are independent of the duration of electrofocusing and which are comparable between experiments and between laboratories.

Chrambach's section has for several years been involved in an attempt to extend the benefits of "quantitative gel electrophoresis" with regard to the identification, physical characterization and efficient separation of particles from macromolecules to virus-sized particles. Last year's advances along that front included the demonstration that crude virus extracts, as well as purified viruses, yielded the characteristic nonlinear "Ferguson plots" on which the method rests. Furthermore, apparatus and procedures were introduced which allow for such analysis at the nanogram level, rather than the microgram level used previously. This improved sensitivity renders the method potentially useful for detection, separation and physical characterizations of clinically relevant viruses. It has also been useful in providing the first "Ferguson plot" of a biologically important multimeric enzyme, eukaryotic pre-tRNA 5' nuclease.

The development of a nanogram-sensitive agarose gel electrophoresis of virus-sized particles, inclusive of the benefits of electrophoretic auto-concentration of the sample ("stacking"), necessarily included instrumental design and construction. It also included the development of discontinuous buffer systems with sufficient rates of boundary displacement for our purpose, and the finding that commercial large-sized DNA ladders can serve to mark such boundaries. These advances in particle electrophoresis on agarose, using discontinuous buffer systems are of particular importance for developing more efficient preparative procedures for viruses and subcellular particles.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00040-12 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Statistical and Mathematical Studies of Molecular Interactions

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	P. Munson	Mathematical Statistician	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	K. Chen	Visiting Associate	LTPB, NICHD
	E. Rovati	Visiting Fellow	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Jennigan	Volunteer Researcher	LTPB, NICHD
	R. Ball	Volunteer Researcher	LTPB, NICHD
	C. Whitlock	Computer Aid	LTPB, NICHD

## COOPERATING UNITS (if any)

VCBS, NICHD (K. Dixon)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.2

## PROFESSIONAL

2.2

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new "universal" curve fitting method has been developed, which combines the advantages of empirical methods (e.g., polynomials) with those of mathematical modeling. The program estimates the curve shape or template automatically by simultaneous analysis of families of curves. Optimal shifting and scaling factors are obtained by weighted non-linear least-squares curve-fitting with appropriate constraints (e.g., monotonicity, number of inflection points) and sharing of parameters. A computer program ("FLEXIFIT") for fitting families of curves of arbitrary shape was developed and enhanced. It is useful for characterizing non-monotonic, dose-response curves and estimation of relative potency in bioassay and immunoassay. Several important enhancements were made to the underlying algorithm.

The curvature test for non-randomness of residuals was further developed and used to establish a dose-response relationship for serotonin release from rat hypothalamus stimulated by corticotropin releasing factor.

A new theoretical study of alternative models of DNA replication/repair mechanisms was begun, which seeks to distinguish gap filling from replication fork progression as the rate-limiting step in repair of UV-damaged DNA.

Mathematical and statistical tools useful for understanding the molecular basis of ligand-receptor interactions were developed and enhanced. The statistical properties of binding affinity estimates was studied using simulation techniques. An extensive study of optimized experimental design for ligand binding studies was begun. This study will lead to improved efficiency in characterizing complex, cross-reacting receptor systems. The popular "Cheng-Prusoff correction" for estimation of the affinity of unlabeled ligands was shown to be an approximation which can yield misleading results. A mathematically exact expression has been derived, which serves as a correction to the "Cheng Prusoff correction".



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-12 LTPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Characterization of Macromolecular and Cellular Particles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Chrambach Head LTPB, NICHD

Others: L Orban Visiting Fellow LTPB, NICHD  
E. Hahn Guest Associate LTPB, NICHD  
D. Tietz Visiting Fellow LTPB, NICHD

COOPERATING UNITS (if any)

USDA, Beltsville, MD (S. S. Hurtt; Human Genetics Branch, NICHD (M. A. Zasloff)  
Lab. Molecular Immunology, NICHD (J. B. Robbins)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.5

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

(1) Ferguson plot analysis of turnip crinkle virus contained in a crude leaf extract reveals a gel concentration dependent size and shape distribution similar to that of the purified virus. (2) A 6 x 8 nm particle from *X. laevis* with 5' pre-tRNAase activity can be stacked and resolved in agarose gel electrophoresis at pH 6. (3) Meningitis immunogen prepared by crosslinking with tetanus toxoid upon 2-dimensional agarose gel electrophoresis yielded a polydisperse gel pattern to be evaluated densitometrically and computationally to yield a size- and charge-profile. (4) Polystyrene sulfate size standards (Interfacial Dynamics Corp.) do not exhibit apparent compressibility up to 1% agarose concentration. (5) Two dimensional electrofocusing-gel electrophoresis yielded titration curves of several plant viruses.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00171-11 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Electrophoretic Methodology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Chrambach	Head	LTPB; NICHD
Others:	J. S. Fawcett	Visiting Scientist	LTPB, NICHD
	L. Orban	Visiting Fellow	LTPB, NICHD
	D. Tietz	Visiting Fellow	LTPB, NICHD
	E. Hahn	Guest Associate	LTPB, NICHD
	M. Buttermann	Guest Worker (Summer Student)	LTPB, NICHD

## COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH (C. Merrill)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Macromolecular Analysis

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

2.25

## OTHER

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

(1) Electrofocusing on immobilized pH gradients in presence of carrier ampholytes (ICAPG) was adapted to 2-dimensional application on vinyl-silanized thin glass strips. (2) Failure of large proteins to focus in ICAPG was remedied by optimizing the proportions of carrier ampholyte and Immobiline. (3) Reproducibility of IPG gels was improved by construction of a mechanized gradient maker. (4) Multicomponent protein mixtures were applied to 2-dimensional gels with the purpose of determining the degree of transfer from ICAPG. (5) The overall-linearity of Ferguson plots of polystyrene sulfate particles on 30% Bis-cross-linked polyacrylamide gel was demonstrated. This suggests that polyacrylamide structure over a wide concentration range is constant, by contrast with agarose. The mechanical instability of the gels renders the method impractical for subcellular particles. (6) Ferguson plots of polystyrene sulfate particles on a fluid medium comprised of 30% Bis-crosslinked polyacrylamide below 2 %T in the presence of 22.5% Metrizamide and 6 M urea are convex, indicating that the determination of free mobilities by linear extrapolation of Ferguson plots of PAGE to 0 %T is inaccurate. (7) Moving boundaries in agarose gel electrophoresis with rapid displacement rates can be marked by a commercial ethidium bromide labeled DNA-ladder. This allows for Ferguson plots of subcellular particles at low gel concentrations, as required for free mobility determination. (8) An improved device for the measurement of field strength across arrays of gel cylinders at various gel concentrations was constructed. (9) Discontinuous agarose gel electrophoresis on horizontal 0.5 mm thin gel strips was developed for Peltier-cooled apparatus. (10) The intersection of linear Ferguson plots of proteins in PAGE based on absolute mobility measurements, using gels of various degrees of crosslinking, was investigated to validate present free mobility estimates of proteins in PAGE.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00189-06 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer Programs for Analysis of Laboratory and Clinical Data

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Rodbard Head LTPB, NICHD

Others: P. Munson Mathematical Statistician LTPB, NICHD  
A. Genazzani Visiting Fellow LTPB, NICHD  
V. Guardabasso Volunteer Researcher LTPB, NICHD  
J. E. A. McIntosh Volunteer Researcher LTPB, NICHD

COOPERATING UNITS (if any) DCRT DMB (B. Cole); Univ. of Virginia School of Medicine (J. Veldhuis); The Penn. School of Medicine (K. Oerter); Kantonspital, Winterthur, Switzerland (R. Lutz); Institute of Pharmacology "Mario Negri", Milan, Italy (V. Guardabasso)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.1

## PROFESSIONAL

1.1

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed new approaches for analysis of episodic hormone secretion in man, experimental animals, and in in vitro perfused cell systems. These methods are statistically valid, objective, reliable, sensitive and yield new physiological information including the instantaneous rate of hormone secretion (ISR) and the half-life or decay constant(s) for hormone metabolism and degradation.

Other computer programs have been developed, including improved methods for analysis of enzyme-substrate-inhibitor systems, radio-receptor assays, and families of multi-exponential decay curves as arise in studies of hormone clearance, receptor dissociation and X-ray inactivation. We have applied these programs to study the dynamics of LH and testosterone secretion in normal men, in men with idiopathic hypogonadotropic hypogonadism, and in men following surgical orchiectomy, with and without testosterone replacement therapy. We find: 1) The frequency of episodes of LH secretion is higher than previously appreciated; 2) tonic secretion of LH is almost negligible; 3) peak duration and interpulse interval are shorter than previously appreciated; 4) the ratio of amplitude/nadir is much higher for LH-ISR than for LH plasma levels; 5) Patients with idiopathic hypogonadotropic hypogonadism almost uniformly have a very high frequency of very small LH pulses; 6) Patients with orchiectomy have high frequency, high amplitude LH secretion, which returns only partly to normal during conventional testosterone replacement therapy. We have developed objective statistical methods to evaluate the coincidence of peaks (pulses) in two hormonal time series, which will be useful in the study of both male and female reproductive biology. Both theoretical and Monte Carlo computer simulation studies indicate that the "DETECT" algorithm and program developed in this laboratory is more sensitive than previously available methods.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-05 LTPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Clinical Applications of Stable Isotopes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. L. Yergey Head LTPB, NICHD

Others: Nancy Vieira Biologist (Tech.) LTPB, NICHD

COOPERATING UNITS (if any) HGB, NICHD (J. Sidbury); Lab. Math. Biol., NCI (D. Covell); Dept. Ped. U. MO Med. Schl., Columbia, MO (L. Hillman); Dept. Endoc., Mayo Clinic (R. Eastell); Cin. Child. Hosp. (B. Specker); USDA, Beltsville, MD (Claude Viellon); Dept. of Nutr., U. Conn. Storrs (Linsay Allen); Haifa, Israel (Zeev Hochburg)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic and Mass Spectroscopy

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

(1) Thermal ionization mass spectrometric analysis of calcium tracers is sufficiently sensitive to have allowed studies of whole body calcium distribution dynamics to be conducted over a six week period following the administration of an intravenous tracer at  $^{42}\text{Ca}$  levels of about 1.2 mg/kg. These studies have been performed in normal lactating women [n=4], osteoporotic woman [1], and patients with end organ vitamin D resistance [2]. Modeling analysis of several of these studies is near completion and shows that a fourth component of the distribution kinetics can be identified with reliability estimates of the same order (about  $\pm 10\%$ ) as the more rapidly turning over components which had been identified in earlier, shorter studies. This component of the distribution is almost certainly associated with a deeper bone turnover than has been previously identified directly by any analytical technique. (2) Premature infants [n=6] show fractional absorption of calcium from dietary sources that are in the range of normal adult values, but which have an absorption rate that is about twice that of normal children (age >4 yrs) and adults. (3) Studies of fractional absorption of calcium in normal adult women show no age dependence in total absorption, but exhibit evidence for an age dependence of absorption rate. (4) Studies have begun which will be used to define dietary recommended daily allowance (RDA) values of calcium and zinc for lactating women. A significant aspect of studies (1)-(4), above, is that these results were obtained by one day studies involving only urine collections. The results from these studies are consistent with the generally accepted methodology of metabolic balance which requires a costly 15 day hospitalization in a metabolic ward of a clinical research center.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01401-05 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biological Applications of Thermospray Liquid Chromatography/ Mass Spectrometry

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. Yergey Head

LTPB, NICHD

Others: N. Esteban Visiting Scientist  
D. Vicchio IRTA  
P. Smith NRCLTPB, NICHD  
LTPB, NICHD  
LTPB, NICHD

COOPERATING UNITS (if any) Div. of Ped. Met., Dept. of Ped., Duke Univ., Durham, NC (D. Millington and C. Roe); HGB, NICHD (J. Sidbury); DEB, NICHD (L. Loriaux and T. Loughlin); LDN, NICHD (D. Brennenman); NCI P-Navy MOB (J. Mulshine, T. Treston)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Metabolic Analysis and Mass Spectroscopy

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

2.5

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

(1) Daily cortisol production rates (PR) have been determined in normal subjects [n=9], in 5 patients with Cushing's syndrome, and 3 adrenalectomized patients using 9,12,12-trideutero-cortisol infused to a steady state using isotope dilution thermospray liquid chromatography/mass spectrometry (ThLC/MS). The method was validated in adrenalectomized subjects by showing observed values for production rate was in perfect agreement with the ratio of infused natural and labelled cortisol. PR measurements in normals yield an average value of  $8.8 \pm 2.3$  mg/24 hours, which is about 50% lower than the previously accepted value. The anticipated circadian variation in PR was observed. Measurements in Cushing's syndrome show PR elevation of 2-3 fold above normal and exhibit a partial to complete loss of circadian variation. (2) ThLC/MS of a wide variety of steroids and their glucuronides along with chromatographic separations have been obtained. (3) Glucose production rates in 6 subjects with Glycogen Storage Disease Type I and unmeasurable glucose-6 phosphatase activity, as determined from enzyme activity of liver biopsy specimens, have been shown to have residual glucose production at levels of about 30% of normal. In addition, these studies suggest a novel approach to maintenance of metabolic balance of the subjects by adjusting dietary glucose intake to levels that suppress elevated plasma lactate levels to normal values.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01404-04 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Rodbard Head LTPB, NICHD

Others: S. Schwarz Chemist LTPB, NICHD

A. Katki Chemist LTPB, NICHD

G-Z. Zhou Courtesy Associate LTPB, NICHD

D. Lichtstein Visiting Scientist LTPB, NICHD

## COOPERATING UNITS (if any)

Dept. Endocrinology, U. Florence (M. Maggi, M. Serio, M. Pazzagli); Rockefeller Univ. (P. Morris)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.8

## PROFESSIONAL

1.8

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have characterized a number of receptor systems for drugs, hormones and neurotransmitters in brain, endocrine glands and reproductive tract. We have demonstrated V1 receptors for vasopressin in male genital tract, especially seminal vesicle, from several species including man. The "oxytocin" receptors in female genital tract (rabbit) were re-examined: in addition to oxytocin receptors in myometrium, there are vasopressin receptors in vagina, uterus and oviduct. Both types of receptors are modulated by sex steroids, pregnancy and delivery, but the nature of the modulation is distinct. These studies are relevant to the mechanisms of normal initiation of labor, premature labor, and the use of oxytocin and other hormones for the induction of labor.

Sensitivity to inorganic anions and cations was used to characterize the opioid receptors of rat brain and bovine adrenal medulla. Further, quantitative ligand binding studies were used to demonstrate that "sigma" receptors for (+)-SKF 10,047 (N-allyl-normetazocine) are distinct from the binding sites for phencyclidine (PCP) in rat brain, in a complex highly cross-reactive system.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01405-03 LTPB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Millitus

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Rodbard Head LTPB; NICHD

Others: P. J. Munson Statistician LTPB, NICHD  
M. L. Jaffey Guest Researcher LTPB, NICHD  
S. Sadikario Volunteer Researcher LTPB, NICHD  
P. Estacio Volunteer Researcher LTPB, NICHD

COOPERATING UNITS (if any) CSL, DCRT (D. Syed, N. Popov); Albert Einstein College of Medicine, Diabetes Research and Training Center (R. Mazze, P. Estacio)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.2

## PROFESSIONAL

0.2

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

We are continuing to develop and refine computer programs to assist physicians, diabetes educators, other paramedical personnel, and patients with intensive insulin therapy. The programs provide analysis of the ambulatory glucose profile, using manual entry and/or verified data from recording or "memory" glucose reflectance meters. Graphical and statistical displays are accompanied by detailed written interpretation, with advice and explanations for alterations of insulin dosage, timing, type, or dietary changes. Nonparametric statistics and principles of exploratory data analysis are combined with techniques for transformation, weighting, smoothing and interpolating, using newly developed, original statistical methods. The programs also provide analysis of patient compliance, and have potential educational value. To avoid the need for tedious manual data entry by typing, the programs accept data automatically from glucose meters equipped with clock calendar and memory. Newly developed logic converts "clock-time" to relationship to meals, permitting the construction of a meal-time glucose profile. The techniques of artificial intelligence (expert systems) is being applied, to facilitate development of flexible reasoning and explanation systems for a wide variety of insulin treatment regimens. This should facilitate the customizing of the "algorithms" by the physician for individual patients.



OFFICE OF THE SCIENTIFIC DIRECTOR

- Z01 HD 00093-13    Mechanism of Action of Nerve Growth Factor  
                            Gordon Guroff, Ph.D.
- Z01 HD 00137-13    Regulation and Expression of the UDP Glucuronosyltransferase  
                            Gene Family  
                            Ida S. Owens, Ph.D.
- Z01 HD 01500-05    Adenovirus (AD) and SV40: Molecular and Cellular Biology  
                            Arthur S. Levine, M.D.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 HD 00093-13 OSD

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Mechanism of Action of Nerve Growth Factor

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Guroff Head OSD:NICHD

Others: G. Dickens	Bio. Lab. Tech.	K. Fujita	Adj. Scientist
P. Lazarovici	Vis. Assoc.	M. Oshima	Adj. Scientist
T. Hama	Vis. Assoc.	J. Tanner	Fed'l. Jr. Fel.
P. Contreras	IRTA	T. Mackey	Summer Student
S. Koizumi	Vis. Fel.		
M. Tocco	Vis. Fel.		

COOPERATING UNITS (if any) Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel  
Department of Biology, Georgetown University, Washington, D.C.  
Laboratory of Cell Biology, NIMH  
Endocrinology and Reproduction Research Branch, NICHD

## LAB/BRANCH

Office of the Scientific Director

## SECTION

Section on Growth Factors

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

7.5

## PROFESSIONAL:

6.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Nerve growth factor (NGF) is a polypeptide required for the survival and development of sympathetic, sensory, and certain central nervous system neurons. It binds to specific cell surface receptors on these neurons, initiates a chain of intracellular events, and, through these intracellular actions, controls the expression of specific genes. The molecular mechanisms by which the factor controls gene expression are not known, but, through the work in this and other laboratories, the cascade of intracellular events mediating the actions of the factor are becoming clear. The binding of nerve growth factor to its receptor on the NGF-responsive cell line, PC12, is followed rapidly by an activation of phosphoinositide metabolism and an activation protein kinase C. This activation appears due to a phosphorylation of the protein kinase C. Kinase C and other kinases lead to changes in the phosphorylation of a number of key proteins in the cell with resultant changes in their activity. Among these are two proteins involved in protein synthesis, the S6 protein of the ribosomes and the elongation factor 2 (EF-2) in the cytoplasm; also altered is the phosphorylation of a nuclear protein (SMP), perhaps proximally involved in gene transcription. We have developed cell-free systems for the phosphorylation of these three proteins in order to dissect the biochemical mechanisms by which the phosphorylations are altered. A major effort now is to find that single reaction coupling the receptor to these kinase pathways. This will be aided by the finding, in this laboratory, of an inhibitor, K-252a, that is specific for the actions of nerve growth factor. It has been shown, for example, that the induction of certain oncogenes, notably c-fos, by nerve growth factor is prevented by K-252a, but induction of c-fos by other ligands is unaffected. Alterations in other gene products are also being explored in order to find out which gene products and in which order are required for the alterations in differentiation produced by nerve growth factor. We have found that nerve growth factor causes the appearance of a characteristic neuronal marker, binding sites for tetanus toxin, that must require the expression of certain glycoprotein genes. We have also found that nerve growth factor treatment causes a decrease in the number of receptors for epidermal growth factor, a mitogen for these cells. An understanding of the mechanism by which the differentiating agent causes a decrease in the receptors for the mitogen could provide an important insight into the overall control of differentiation and cell division.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00137-13 OSD

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

REGULATION AND EXPRESSION OF THE UDP GLUCURONOSYLTRANSFERASE GENE FAMILY

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	I. S. Owens	Head	OSD, NICHD
Others:	O. Michioka	Visiting Fellow	OSD, NICHD
	V. McCauley	Biological Aid	OSD, NICHD
	J. Ritter	Intramural Research Training Award Fellow (IRTA)	OSD, NICHD

## COOPERATING UNITS (if any)

D.W. Nebert & coworkers, Section on Pharmacogenetics, LDP:NICHD:NIH  
G. Hawsworth, Department of Medicine & Therapeutics, University of Aberdeen,  
Aberdeen, Scotland

## LAB/BRANCH

Office of Scientific Director

## SECTION

Section on Drug Biotransformation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.3

## PROFESSIONAL

2.75

## OTHER

.55

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The regulation of the family of UDP glucuronosyltransferase enzymes is being studied at the level of RNA, DNA and protein chemistry. Transferase activities toward certain substrates are known to be induced by different types of effector compounds; such compounds used in these studies include phenobarbital (PB), 3-methylcholanthrene (MC) and clofibrate (CF). We have purified 10-15 different mouse and 6-8 different human transferase cDNA clones by specific antibody reactivity with fusion proteins produced by the appropriate cDNA library constructed in the  $\lambda$ gt11 vector. One of the mouse clones, pUDPGT<sub>m</sub>-5, contains a 2.4 Kb insert and encodes an MC-regulated mRNA. The cDNA has been nearly completely sequenced and has been used as a probe to isolate the corresponding genomic clones from a mouse genomic library in Charon-4A phage. The genomic clones are being restricted in preparation for sequencing the 5' upstream region and the exon-intron junctions. The mouse cDNA, UDPGT<sub>m</sub>-1, (previously characterized with respect to nucleotide sequence, deduced amino acid sequence, signal peptide, transmembrane spanning region and mRNA regulation) was inserted into two different yeast vectors, transfected into two different host cells, and is shown by Western blot analysis to express a transferase protein ( $M_r = 68,856$ ). Studies are in progress to determine the substrate specificity of the transferase. The expression in yeast should make it possible for us to determine the substrate specificity for each transferase cDNA clone. One human cDNA UDPGT<sub>h</sub>-1 (2.5 Kb) encoding a 2500-nt mRNA (shown to have a high degree of similarity to UDPGT<sub>m</sub>-1) is about 75% sequenced and is being prepared for transfection into yeast for expression studies. The orphan drug (in USA) Zixoryn (flumecinol) administered to newborns in certain countries to prevent neonatal jaundice is shown in our laboratory (in mice and rats) for the first time to induce specifically bilirubin transferase activity.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01500-05 OSD

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenovirus (Ad) and SV40: Molecular and Cellular Biology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A.S. Levine	Head	OSD, NICHD	
Others:	C.T. Patch	Sr. Investigator	E. Roilides	Visiting Fellow
	K. Dixon	Sr. Staff Fellow	M. Carty	Visiting Fellow
	J.M. Hauser	Microbiologist	M. Protic-Sabljjic	Guest Researcher
	A. Razzaque	Sr. Staff Fellow	A. Roy	Guest Researcher
	K. Murai	Visiting Fellow		

COOPERATING UNITS (if any) Lab. of Immunopathology, NIAID (A.M. Lewis, Jr., & M. Carbone); Dept. of Medicine, Natl Jewish Hosp. & Rsch Ctr., Denver (J. Cook); Lab. of Theoretical & Phys. Biol., NICHD (P. Munson); Lab. of Develop. Pharm., NICHD (J. Gielen & D. Nebert); Sect. on Molecular Struc. & Protein Chem., ERRL, NICHD (H. Chen); Lab. of Molecular Genetics, NICHD (R. Miskin)

## LAB/BRANCH

Office of the Scientific Director

## SECTION

Section on Viruses and Cellular Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

8.0

## PROFESSIONAL:

6.5

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chromosomal mutations are the underlying cause of most inherited diseases and many developmental abnormalities. Mutations can also lead to alterations in gene expression in somatic cells, leading to loss of the normal differentiated phenotype and ultimately to cellular transformation. We are studying the mechanism of mutagenesis, using an SV40-based shuttle vector as a probe to investigate the molecular mechanisms by which agents that damage DNA induce mutations in mammalian cells. Through use of the shuttle vector, we have extensively characterized the types of mutations that occur in mammalian cells either spontaneously or in response to DNA damage. Analysis of the sequence specificity of these mutations has led to a model which explains how the mammalian DNA polymerase introduces errors during DNA synthesis, causing mutations. Studies with the vector in an in vitro DNA replication system indicate that cellular factors, in addition to DNA polymerase, appear to influence replication fidelity. Further studies with this system should allow a characterization of these factors on the biochemical level.

Understanding the mechanisms of regulation of cellular proliferation and differentiation is basic to understanding development of multicellular organisms. One approach to investigating these regulatory mechanisms is to study the behavior of transformed cells. For the past several years, we have been studying mitogenic and antimitogenic growth factors secreted by hamster cells transformed by Ad2 and SV40. Our findings indicate that SV40-transformed cells, but not Ad2-transformed cells, secrete a mitogenic inhibitor (MI) that strongly inhibits a proliferative response in untransformed hamster cells and normal rat cells stimulated with serum mitogens. MI also inhibits a mitogenic response by normal hamster spleen lymphocytes stimulated with lectins that activate T cells (concanavalin A) or B cells (pokeweed mitogen). We have proposed that MI might contribute to the high oncogenicity of the SV40-transformed cells by interfering with mobilization of immune effector cells at the site of tumor growth. We are also using SV40 to study the genetic basis of viral tissue tropism. We find that subcutaneously injected small t-antigen mutants of SV40 often induce abdominal lymphomas in hamsters, rather than the subcutaneous fibrosarcomas induced by wild-type SV40. The mutants may fail to produce a growth factor required for the in vivo transformation of non-proliferating cells.



P R E V E N T I O N   R E S E A R C H   P R O G R A M

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Office of the Director, Prevention Research Program

We changed our name from Epidemiology and Biometry Research Program to Prevention Research Program to reflect the additional responsibilities in the coordination of prevention research activities of the NICHD as required by the Health Research Extension Act of 1985, PL 99-158. This legislation provided for the position of Associate Director for Prevention Research who will develop and conduct research in health education and disease prevention in the field of maternal and child health and in the areas of family planning and population. The staff and research activities of the Epidemiology and Biometry Research Program have become part of the Prevention Research Program with the Director of the Program also serving as Associate Director for Prevention Research, NICHD.

The Program will continue its epidemiological and biostatistical investigations and also clinical trials in the areas of maternal child health, reproductive and perinatal epidemiology, low birth weight intrauterine growth retardation and pre-term delivery and also infant feeding practices and its effect on growth and development. In addition, the Prevention Research Program will initiate and conduct research in health promotion and disease prevention. This includes health behavior during pregnancy such as the avoidance of smoking and drinking and the obtaining of prenatal care and compliance to improve pregnancy outcome, preventive health behavior during childhood and adolescence, which include nutrition appropriate to the age of the child, exercise, behaviors leading to the prevention of teenage pregnancies and the avoidance of behaviors which lead to the risk of acquiring AIDS. Another area is accident and injury prevention which covers the whole span of the pediatric age period including adolescence in an effort to reduce the risk of injury or death from these causes. PRP is presently recruiting a senior investigator who has appropriate expertise to direct this aspect of our program.

The Office of the Director has assumed responsibilities for the development of a protocol for a clinical trial of the use of IV gamma globulin in the treatment of children with AIDS. Since it is anticipated that the antiviral agents in the treatment of children with AIDS will become available sometime in 1988, there is only a limited time available in which to conduct a clinical trial of the efficacy of IV gamma globulin in order to avoid competing for the same children with these treatment modalities. In discussion with the National Institute of Allergy and Infectious Diseases in May of 1987, we offered our expertise in the conduct of clinical trials and

volunteered to take the initiative with their support in expediting the development of this trial and to get it off the ground sometime this summer. The development of the protocol is largely complete and was done with the participation of a few outside consultants. Participating units will be selected from ATU's supported by NIAID who have access to children with AIDS and are interested in participating, with funding to be provided by NIAID, and from a list of hospitals who identified themselves in response to a sources sought announcement which offer an estimated 3-400 children to be available for this trial during the next six months. The trial should be operational in September 1987.

This office is involved in a number of international research projects and activities. The Bedouin Infant Feeding Study is complete and analysis is in progress. The analysis had been delayed because of lack of programming support which has now become available. A somewhat parallel study of infant feeding practices among North African Jews recently immigrated to Israel is in the final phases of data collection. In that study more specific indicators of nutritional status were collected during pregnancy and children were followed through 30 months of age.

The planning for a pregnancy outcome study in collaboration with the Aga Khan University in Karachi is in the final phases. This project will focus on the identification of risk factors associated with birth outcome and the role of intrauterine growth retardation and pre-term delivery in mortality and morbidity in the first two years of life. The project will be conducted in four sites; that is, maternity hospitals supported by the Aga Khan Foundation in Karachi serving a middle class population, Katchi Abadis which are slum areas in the outskirts of Karachi, rural sites in the province of Sind and a rural site in the mountainous area in the northern part of Pakistan. In a cross sectional study in the Katchi Abadis recently conducted, there were 9 maternal deaths among 350 births for a rate of about 26 per 1,000 providing an indication for the high risk status of this population at a par with other developing countries.

The Office of the Director with the Office of the Director for the Center for Research for Mothers and Children is planning a workshop in conjunction with the Indian Medical Research Council and the All-India Institute of Medical Research in New Delhi on Perinatal Determinants of Child Survival sometime in the early part of next year. The focus of the workshop will be the identification of interventions which appear feasible and focus on the reduction of infant mortality, low birth weight or pre-term delivery in India. Areas under consideration are screening programs administered by para-medical personnel to identify during pregnancy women with conditions requiring referral to properly staffed medical centers



and the organization of such a referral system, screening for infectious diseases during pregnancy and their treatment, screening to identify under- and malnutrition and appropriate interventions, the prevention of neonatal tetanus, and the role of neonatal intensive care if any.

We have also recently been involved in consultation with the Agency for International Development in regards to their new maternal and perinatal health and nutrition program.

In collaboration with the OD/CRMC and the Division of Maternal Child Health, HRSA, we will develop a conference on clinical advances in the prevention of low birth weight as a followup to the conference in Evian sponsored by Dr. Papiernik in May of 1985. This conference will bring together individuals who have recently completed clinical trials or community based interventions aimed at the prevention of low birth weight be it intrauterine growth retardation or pre-term delivery.

Also in the planning stages is a workshop bringing together individuals who have done research with the tokodynamometer to review the state of knowledge which has been assembled in these investigations in order to identify needs for further research, if any.

Other research activities in which the OD/PRP is involved have been summarized under the Epidemiology and Biometry Branches.

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Office of the Director, PRP

Publications

Berendes, H.W.: Current issues in perinatal epidemiology. Yale Journal of Biology and Medicine 60: 349-358, 1987.

## BIOMETRY BRANCH

- Z01 HD 00801-12 Studies Based on the Medical Birth Registries of  
Norway and Sweden  
H. J. Hoffman
- Z01 HD 00802-12 Studies of Linked Live Births-Infant Deaths and  
Fetal Deaths from U.S. States  
H. J. Hoffman
- Z01 HD 00803-03 Analysis of Sudden Infant Death Syndrome (SIDS)  
Risk Factors  
H. J. Hoffman
- Z01 HD 00811-08 National Collaborative Cysteamine Study Data Center  
G. F. Reed
- Z01 HD 00813-06 Biostatistical Methods for Laboratory Research Studies  
G. F. Reed
- Z01 HD 00818-06 Research in Developing Nonparametric Methods for  
Biomedical Applications  
G. F. Reed
- Z01 HD 00820-06 Statistical Methods for Epidemiologic Data  
D. W. Denman
- Z01 HD 00821-05 Development of New Graphical Methods for the Analysis  
of Biomedical Data  
D. W. Denman
- Z01 HD 00840-06 Statistical Discriminant Methods with Applications  
to Alcoholism Screening  
B. I. Graubard
- Z01 HD 00841-06 Methods for Comparing and Analyzing Data from  
Several Complex Surveys  
B. I. Graubard
- Z01 HD 00842-05 Development of Statistical Methods to Analyze  
Cluster Samples  
B. I. Graubard
- Z01 HD 00843-04 An Investigation of Matched Analysis in Case-  
Control and Cohort Studies  
B. I. Graubard



- Z01 HD 00850-11 Randomized, Controlled Study of Phototherapy for  
Neonatal Hyperbilirubinemia  
D. A. Bryla
- Z01 HD 00852-05 1980 National Natality Survey and Fetal Mortality Survey  
D. A. Bryla
- Z01 HD 00853-03 Design and Analysis of a Clinical Trial of Vi Poly-  
saccharide Vaccine  
D. A. Bryla
- Z01 HD 00854-03 Analysis of MCH Data from the National Logitudinal  
Youth Survey  
D. A. Bryla
- Z01 HD 00860-07 Analysis of Biomedical Time Series Data  
H. J. Hoffman
- Z01 HD 00861-05 Assessment of In-Utero Fetal Growth Patterns in  
Relation to Outcome at Birth  
H. J. Hoffman
- Z01 HD 00870-04 Long-Term Reproductive Effects of Cesarean Section Birth  
B. I. Graubard
- Z01 HD 00871-02 Clinical Trial of New Drug Therapy for Cystinosis  
G. F. Reed
- Z01 HD 00872-02 Factors Associated with Premature Births:  
Missouri Follow-back Survey  
D. A. Bryla
- Z01 HD 00873-01 Relationship of Mother's Prepregnancy Size to Pregnancy  
Complications and Outcome  
B. I. Graubard

NICHD ANNUAL REPORT  
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Biometry Branch

The Biometry Branch research activities are structured along three lines: (1) provision of statistical analysis and consultation to NICHD Intramural and Extramural investigators; (2) pursuit of individual and collaborative research in biometry, including both mathematical and biostatistical theory and applications; and (3) support of clinical trials initiated by the NICHD. The Branch maintains strong ties to both the Intramural and Extramural research programs of the Institute. Also, the Branch has supported a number of cooperative studies, including projects supported solely by NICHD and those receiving joint funding from other agencies within the U.S. Public Health Service.

The following review of Biometry Branch research activities is organized by subject matter, rather than by the statistical or mathematical methods utilized in the planning, design, conduct, or analysis phases of these research efforts.

Perinatal Morbidity and Mortality

Perinatal morbidity and mortality are key outcome variables for several studies being performed by the Biometry Branch. A major effort has been devoted to studies comparing United States data with that of two population-based perinatal data sets from Scandinavia, the Medical Birth Registries of Norway and Sweden.

In general, perinatal mortality rates provide a better indication of the availability, utilization, and effectiveness of health care for the pregnant woman and her fetus than the more traditional index of infant mortality. A recent publication based on data from four Nordic countries, 1900-1980, showed that "infant" mortality is a sensitive indicator of changing socio-economic circumstances, but perinatal mortality rates are more responsive to changes in underlying demographic factors--maternal age, parity and spacing between births--and to changes in the prenatal, obstetric, and pediatric care provided. A study currently underway in conjunction with the Office of International Statistics, National Center for Health Statistics is examining recent trends in perinatal and infant mortality rates over the past decade and a half for the six countries participating in the International Collaborative Effort on Perinatal and Infant Mortality (ICE). The birth weight-specific comparisons used in this study provide documentation for the probable impact of such technological developments as neonatal intensive care units since 1970. For international comparison of

birth weight-specific perinatal mortality rates, we have shown that it is necessary to adjust for any differences in underlying birth weight distributions between populations in different countries before inferences can be made. However, for comparison through time within a country, birth weight distributions are sufficiently stable to make direct comparisons of birth weight-specific perinatal mortality rates.

Based on the ICE data, the perinatal mortality rate in the United States declined 43% for blacks and 49% for whites from 1970 through 1983. Comparable percentage improvements in perinatal mortality rates occurred among the other participating countries in ICE during the same time period: England & Wales, 56%; Japan, 57%; Norway, 50%; Scotland, 57%; and Sweden, 58%. When birth weight-specific perinatal mortality rates are compared over time there is a nearly uniform improvement on a logistic scale for each 500-gram weight category (from 500g through 4500g or more) during this time period in the United States. However, on closer inspection, it is clear that late fetal mortality rates have improved at a faster rate in the normal weight range, 2,500g and above, whereas the early neonatal mortality rate has improved relatively more for low birth weight infants, less than or equal to 2,500g. The analysis of birth weight-specific perinatal mortality rates over the same time period in Japan revealed that the greatest relative improvement occurred in the normal birth weight range, 2500g and above. Conversely, in Norway and Sweden the greatest relative improvement during this time period occurred in the low birth weight range and, especially, in the very low birth weight range, less than 1500g. These different patterns in the relative improvement in birth weight-specific perinatal mortality rates in Japan versus Norway and Sweden reflect the fact that late fetal mortality rates have improved relatively more in Japan while early neonatal mortality rates have improved relatively more in Scandinavia during this time period.

Another study, a secondary analysis of previously unpublished data obtained from the National Center for Health Statistics, has been undertaken to review changes in perinatal and infant mortality by race in selected U.S. cities. Data from the current NICHD/NIEHS Study of Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates will also be used in assessment of recent time trends. This latter study is being carried out through the contract mechanism with the Departments of Health of five U.S. States--Michigan, Missouri, New York (Upstate), North Carolina, and Utah--and in four foreign countries--Australia (three States), Japan (Osaka province), Norway, and Scotland. A uniform data tape format has been developed for the years 1980-84 and each contractor is preparing their data according to this format. A uniform procedure for classifying the "level" of medical care at birth has been achieved for the five U.S. States. The foreign participants will



also define "level" of medical care in three broad categories (I, II, or III). One of the principal aims of this study will be to compare the perinatal mortality attributable to "preterm" low birth weight infants in contrast to "SGA" low birth weight infants in the U.S. and each of the foreign countries. Standards defining the 10th percentile of birth weight-for-gestational age in each of the relevant population groups or subgroups are being developed. Each data set will contain some descriptors of education or occupation of parents so that some social factors can be controlled for. Detailed cause-of-death (ICD-8 or ICD-9) information will also be available for study.

One research effort that has emerged out of this general interest in perinatal morbidity and mortality is a prospective study designed to delineate risk factors for fetal growth retardation. Retarded fetal growth, defined as a birth whose weight is below the 10th percentile of birth weight-for-gestational age, is associated with increased rates of both perinatal mortality and morbidity. Using the research contract mechanism, this prospective study is being conducted at two locations: the University of Alabama in Birmingham and the University of Trondheim, Norway. The latter project also includes subcontracts with the University of Bergen, Norway and University of Uppsala, Sweden to supply additional data based on pregnancies and deliveries in these areas.

The aim of this research project is to determine risk factors which will distinguish mothers who have repeated small-for-gestational age (SGA) births from those mothers who have a single, unexpected SGA birth. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally via diagnostic ultrasound measurements and at delivery with standardized measurements. The study protocol includes recruitment of pregnant women before 17 weeks gestation and subsequent enrollment of women with high risk pregnancies through 33 weeks of gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy.

Pregnant mothers have been enrolled in this study since November 1985 with deliveries of newborn infants since April 1986. The plan is to continue enrolling pregnant mothers through March 1988 in order to enroll 1500 women into this prospective study in both Alabama and Scandinavia. At that time, we estimate that approximately 300 SGA births will have occurred in both the Alabama and Scandinavian sites. Study infants will be followed-up throughout the first year of life to assess catch-up growth, to monitor breast or bottle feeding patterns and occurrence of illnesses, and to assess the achievement of developmental milestones.

Perinatal mortality and morbidity data are also being examined in several other statistically diverse projects. Data from a variety of sources are being analyzed in different ways to study birth outcomes such as birth size, prematurity, and mortality. In collaboration with a former Visiting Scientist from Sweden, a matched case/control study has demonstrated that there is no adverse effect of a previous induced abortion on gestation or birth weight in the subsequent pregnancy, unless there had been a medical complication with the abortion. Another study has compared the outcome of deliveries of women who conceived with an IUD in place to those without an IUD present using a data set derived from the Kaiser Permanente Birth Defects Study. Also, in collaboration with Norwegian scientists, a data base of extensive longitudinal antenatal measurements, for example, symphysis-fundal heights, ultrasound measurements, hemoglobin, maternal weight-gain, and smoking, have been related to weight and length at birth in a Norwegian cohort. These results have produced information which may be useful clinically in assessing high risk pregnancies. In another research study conducted jointly with a Visiting Scientist from Norway, "precise" gestational age values have been determined for various subgroups within a large Scandinavian population of births.

The Biometry Branch is also working on research studies based on the 1980 National Natality Survey and 1980 National Fetal Mortality Survey conducted by the National Center for Health Statistics. The available data base is comprised of 9,941 live births and 6,386 fetal deaths. Initial maternal blood pressure readings during pregnancy have been analyzed in relation to a number of variables including birth outcome, maternal race, education, and age. An additional study is examining a possible relationship between maternal obesity and increased use of cesarian section for delivery. Because of the small number of very low birth weight (VLBW) infants, <1500 grams, included in the 1980 National Natality Survey, we have undertaken a new research contract study to be conducted in Missouri. Information will be obtained through mailed questionnaires to study mothers including all mothers of VLBW infants, all mothers of fetal deaths, a sample of mothers with moderately low birth weight infants (between 1500-2499 grams), and a sample of mothers with normal birth weight infants (>2500 grams). Data will also be obtained from vital records and medical records abstraction. Study infants will be assessed with a developmental screening test at one year of age.

#### Phototherapy Treatment for Neonatal Hyperbilirubinemia

Since 1974 the Biometry Branch has actively participated in the conduct of this clinical trial. This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by

comparing treated with untreated infants under specific conditions. The Biometry Branch has served as the data center for this study, and was the focal point for receipt of 1,339 newborn examinations and approximately 1,000 follow-up examinations per year until the children were six years of age. These forms were checked for accuracy, and precoded at each data collection center. The master files for each year's follow-up have been edited for keypunch and coding errors, and for internal consistency. The Branch has also been responsible for the analysis of the data.

During this year intensive effort has been exerted by a special working group to review the records of all the neurologically suspicious and abnormal cases of the one and six year examinations. The ultimate purpose of this review is to determine if there are any significant differences between the children treated with phototherapy and those that did not receive this treatment. If there are any significant differences the data will be analyzed to determine if bilirubin level and/or treatment modality is related. As part of the neurological examination, timed movements, such as heel/toe and hand pats, were tested at six years. Presently, this data is being analyzed to see if there are any differences by sex, race or birth weight. It is anticipated that two manuscripts will be ready for submission to journals by the end of the year.

#### Sudden Infant Death Syndrome (SIDS) Risk Factors

A major effort of the Branch has been invested in support of the NICHD Cooperative Epidemiological Study of the Sudden Infant Death Syndrome (SIDS) Risk Factors. All of the data collected for this study have been edited and entered onto computer files by staff of the Department of Biostatistics, School of Public Health and Community Medicine, University of Washington, Seattle. With final results of the pathology second review now available, we can state that SIDS was the final classification for 94.6% of the singleton, non turn-around infants submitted to the Pathology Coordinating Laboratory as having died of SIDS by the local medical examiners or coroners. Another 2.3% were classified as "possible" SIDS cases, while 1.4% were impossible to determine due to missing materials or vital information. Only 1.8% of the eligible SIDS infants were determined by the Pathology Review Panel to have died of "known" causes and were, therefore, non SIDS.

Based on the analysis of the 757 pathologically-defined singleton SIDS cases and both sets of matched, living controls (1,514 singleton living infants), the following descriptive information has emerged. Overall, 54 percent of the SIDS cases were black, although only 26 percent of the births in the study centers were black. Slightly less than 5 percent of SIDS cases were multiple births, but this represents an increased risk of two and one-half times greater



than that for singleton births. The percentage of low birth weight infants is increased among cases by almost four-fold over random control infants (Controls A's). As expected because of matching, the percentage of low birth weight Control B infants is nearly identical to that of cases. The comparison between black and non-black infants revealed substantially higher rates of low birth weight among blacks, both for cases and Control A's.

Aggregate data on births and infant deaths in 1979 were provided by the National Center for Health Statistics so that the total incidence in our study areas could be compared with national figures. The national data agree quite well with the non-black SIDS incidence figures for the NICHD SIDS Cooperative Study at 1.1 to 1.3 per 1,000 live births. However, marked variations did occur between the different study centers. Moreover, the SIDS incidence among blacks in the NICHD SIDS Cooperative Study was 29 percent higher than indicated by the national data. Further examination of infant and post-neonatal mortality rates by race for the NICHD SIDS Cooperative Study and the national data did not show any substantial differences. Thus, it may well be that SIDS is underreported as a cause of post-neonatal mortality, particularly among black infants in the United States.

During the last few years, some reports have suggested the possibility of a cause and effect relationship between immunization with diphtheria-tetanus-pertussis (DTP) vaccine and sudden infant death. The NICHD SIDS Cooperative Study collected data relevant to this issue both from maternal interviews and from the abstraction of medical records of the study infants. There were no differences found regarding a temporal association between DTP immunization and time of death for SIDS cases and time of interview for control infants. However, significantly fewer cases were ever immunized with DTP (39.8%) as compared to Control B, or Control A, infants (53.2% and 55.0%). This difference may reflect the fact that SIDS parents as a group generally did not have the same access to the medical care system. For example, significantly more SIDS cases (26.1%) received no regular well baby care compared to either set of age-matched control infants (15.3% and 15.6%).

In addition to the results quoted above, a number of expected findings have been confirmed by the NICHD SIDS Cooperative Study:

1. 18.2% of SIDS cases had gestation of less than 37 weeks compared to 4.6% of Control A's;
2. 59.7% of SIDS cases were male infants;
3. 31.5% of SIDS cases were born to women less than 20 years of age as compared to 17.2% for Control A and 21.7% for Control B infants;



4. only 26.6% of SIDS cases were first-born compared to 39.2% and 41.4% of Control A and B infants;
5. maternal smoking during pregnancy was reported in 69.6% of SIDS cases and in only 37.9% and 42.0% of Control A and B infants.

Also, the failure to find associations with a number of maternal variables which were previously suggested in the literature is of interest. Thus, there were no differences found between SIDS case and Control B mothers in the incidence of urinary tract infection, vaginitis, venereal disease or other maternal problems. No significant differences were found in C-section rates, use of maternal anesthesia and/or analgesia, or in the length of stages 1 and 2 of labor. There were no differences in the incidence of delivery complications, placenta previa, or in mean 1 and 5 minute Apgar scores. However, when compared to Control A infants, SIDS infants did have an increase in a number of nonspecific symptoms, including: respiratory distress, tachypnea, apnea of the newborn, tachycardia, cyanosis, pallor, irritability, poor feeding, jaundice, vomiting, abnormal cry, lethargy and tremors. After comparison to the ethnic and birth weight matched control B infants, only tachypnea, tachycardia, cyanosis, and vomiting remained significant.

A special analysis has been performed in regard to apnea, prematurity and growth retardation for an NIH Consensus Development Conference. The results demonstrated that almost all "apnea" in the newborn nursery occurred among preterm infants and there were no differences in the observed rate between SIDS infants and the birth weight- and race-matched control infants (4%). The evidence from the study indicates that more SIDS cases experienced prenatal and post-natal growth retardation compared to control infants. Also, there were more documented post-neonatal episodes of "turned blue or stopped breathing" compared to either group of control infants (7% vs. 3%). These post-neonatal "apneic" episodes were reported by mothers at the time of interview. Only a very small number of these episodes led to emergency room visits or other medical attention. Also, 60% of these reported episodes were noticed only one time (the remainder were reported to have occurred two or more times). These recurrence rates did not differ between case and control infants.

Breast-feeding and SIDS has also been the subject of an analysis completed this past year. SIDS infants were "mostly or only breast-fed" significantly less often than either the Control A or Control B infants ( $p < .001$ ). Logistic regression analysis controlling simultaneously for mother's age, parity, smoking, education, and income resulted in an adjusted odds ratio of 2.0 compared to Control A infants and 1.7 compared to Control B infants. Lack of breast feeding was a stronger risk factor for mothers with higher levels of education compared to those who had not completed high school. Although symptoms of vomiting and rash were reported

more frequently for SIDS infants, this association was independent of feeding pattern. Why this relationship between SIDS and decreased exposure to breast-feeding exists is unclear. Additional research effort will be required to suggest the mechanism.

#### Childhood Diseases or Disabilities

A major commitment of time and attention of the Biometry Branch is the use of the randomized clinical trial and its surrogates in order to advance research goals of the Institute. Concentrated in the Branch is expertise in the design, conduct, and analysis of comparative clinical studies that seek to evaluate the efficacy of therapeutic and preventive interventions. The Branch is nearly always called upon to participate when some group in the Institute contemplates such a study.

An example of one of our efforts in the area is the long-standing commitment to evaluating therapies for nephropathic cystinosis, a rare inborn metabolic disorder characterized by a surfeit of cystine in the body's tissues that interferes with normal body growth and especially attacks the function of the kidneys. Renal dysfunction is progressive and culminates in end stage renal disease usually by age 10-15 years. Known to deplete the cystine content of human leucocytes in vivo, cysteamine was regarded as a candidate drug for retarding or stopping the deterioration of renal function due to cystinosis. This study was directed by a Principal Investigator at the University of California at San Diego and was organized to recruit cystinosis patients nationwide and provide the protocol and drug for their treatment. Data management and analysis was the responsibility of the the Biometry Branch with assistance from the Computer Science Section. Since the beginning of recruitment in 1978, 98 patients entered the study to receive 4 times daily oral doses of the drug for an average length of treatment of 33.4 months. Final evaluation was made this year of cysteamine as therapy for nephropathic cystinosis. The kidney function parameters, level of serum creatinine and creatinine clearance, were the outcome measures used for evaluation. For patients starting treatment with a serum creatinine level no greater than 2 mg/dl and receiving at least one year of treatment, mean end of study creatinine levels were 0.95 for cysteamine group and 1.41 for the control group. Creatinine clearance means were 43.8 ml/min/1.73 m<sup>2</sup> and 27.8 ml/min/1.73 m<sup>2</sup> for the two groups, respectively. Both sets of differences were significant at the .01 level and persisted after adjustment for baseline age, baseline serum creatinine, and age at end of study. These results support the claim that cysteamine can at least retard the course of renal dysfunction. An additional salutary effect of cysteamine is that body growth for those receiving cysteamine was closer to normal than that of the controls.

Although cysteamine has been judged to have a beneficial effect for victims of nephropathic cystinosis, many patients in the National Collaborative Cysteamine Study found the drug's unpleasant smell and taste so repugnant that they were unable to accept the full protocol dosage and, therefore, never received the full potential effect of cysteamine. The existence of another cystine-depleting agent, phosphocysteamine, which is more palatable than cysteamine, but is yet untested as a therapeutic drug, led to the establishment of a new clinical trial to evaluate alternatives to cysteamine. The University of California at San Diego has been contracted to conduct a randomized clinical trial to evaluate the effectiveness of phosphocysteamine relative to cysteamine on at least 80 patients to be enrolled in a 3-4 year period. This study, which is to identify and develop new drug therapies and to test them against cysteamine as a standard, quickly identified phosphocysteamine as the only practical alternative cystine-depleting agent. It was later found that oral phosphocysteamine is biologically equivalent to cysteamine within minutes of ingestion. The final design of the trial, therefore, is to compare the effects of "low" dose phosphocysteamine to those of a higher dose, with a random assignment of doses to patient. Endpoint measures will be growth variables and glomerular function indices: serum creatinine and creatinine clearance.

The measurement of creatinine clearance presents an ancillary problem that will be addressed with the use of data from the Cysteamine Study and the current trial. Clearances are obtained from 24 hour urine collections, which are difficult to draw reliably and accurately from young patients. A surrogate measure, which employs the patient's height and serum creatinine, has been developed for the general population of pediatric renal disease patients, but a method specific to cystinotic nephropathy is necessary in this case. Analysis on a small set of data has shown that a linear regression predictor may adequately substitute for actual creatinine clearance.

The Biometry Branch is participating in the planning and coordination of a clinical trial involving pediatric AIDS patients. The Prevention Research Program is proposing to fund a data center for a clinical trial to evaluate the efficacy of intravenous immunoglobulin (IVIG) to suppress bacterial infections in children with HIV infection. Since it is expected that antiviral agents for AIDS therapy will become available in relatively short time, it is necessary to resolve the matter of the usefulness of IVIG before the widespread use of antivirals precludes a clean, unconfounded evaluation. It is expected that a two year trial will quickly confirm that IVIG will reduce the incidence and severity of serious infections and that characteristics of patients most likely to benefit from the treatment will be evinced. The Biometry Branch is providing expertise in clinical trial design, namely, clarification



on the issues of stratification, blinding, randomization, compliance, sample size and power.

Another area of collaborative research has been in the treatment, detection and risks of abusive drinking. Branch staff have continued to work with intramural researchers from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) on the problems of finding biological markers for abusive drinking, and in characterizing patients who will be effectively treated for alcoholism. Statistical questions which have been addressed include the best way to derive a set of biological markers for detecting abusive consumption of alcohol and to select a discriminant function for the screening of heavy drinkers in a population. Research has been conducted into the robustness of quadratic, linear, and nonparametric discriminant functions and into potential benefits of applying simple rank and inverse normal score transformations to the original data. In addition, research into the best way to analyze repeated measures of biological markers for detecting alcoholism is being done. Monte-Carlo methods have been used to study the statistical properties of normal theory based classical procedures for analyzing repeated measures data from nonspherical non-normal distributions. In this work simple data transformations are considered for improving the performance of the classical methods. Related research efforts include the participation of Branch staff in collaboration with the Epidemiology Branch in studying the risk of malformed babies which are associated with prenatal consumption of alcohol.

Since 1985 the Biometry Branch has collaborated with the Laboratory of Developmental and Molecular Immunity, IRP on the Vi Polysaccharide Vaccine Trial in Nepal. Staff participated in the training of field staff in Nepal, and analyzed the data of the pilot study for safety and immunogenicity. In March 1986, 6,912 participants were vaccinated with either the Vi vaccine or a polyvalent pneumococcal vaccine in double blind format, using syringes filled according to a random number program and coded by the Institute Merieux. At the end of the first year of surveillance, 26 confirmed cases of typhoid have been diagnosed in the participants. An independent monitor for this study determined that six of the typhoid cases were given the Vi vaccine and the other 20 received the pneumococcal vaccine. This is significant with a  $p < .001$ . This trial will last until March, 1988 with visits to the participants every two days to check for fever.

Another study, based on the 1981 Child Health Supplement, has included collaborative data development and analysis with the National Center for Health Statistics to produce reliable national descriptions of children's health. Two papers have been drafted for publication as journal articles on: "The Health Status of Low Birth



Weight Children in the U.S." and, also, "Complications of Childbirth: Self-Reporting from the Child Health Supplement of the National Health Interview Survey Compared to Two Other Surveys." Future analysis plans include a more detailed analysis of the low birth weight children in terms of significant prenatal events and the childrens' later health outcome.

### Growth and Development

A significant amount of Branch staff effort has been in the nutrition and growth area. These efforts first began with the analysis of infant feeding data from the Pima Indian Reservation and the George Washington University Study, and have continued with the analysis of the Bedouin Arab Infant Feeding Study. The Pima Indian and the Bedouin Arab data sets were cluster samples including data on all the children in the family. The proper analysis of clustered data where binary observations within each cluster may be correlated is a statistical problem that has been investigated by Branch staff.

There has been collaboration with staff of the Epidemiology Branch involving several analyses of the first and second National Health and Nutrition Examination Surveys (NHANES I and II). In the process of analyzing the NHANES data it became clear that there were deficiencies in the statistical methodology for the analysis of complex survey data such as NHANES. This resulted in the development of a research contract to develop new methods for doing regression analysis on NHANES. A contract was awarded to the Research Triangle Institute in North Carolina to expand and develop regression methodology for complex surveys that can be applied to the analysis of growth and nutrition relationships in NHANES. This project, entitled Analysis of Relationships between Childhood Growth and Dietary Intake Using NHANES II, has just entered the second year. The work during the second year consists of: (1) finishing the mathematical details relating to the estimation and evaluation of the parameters and test statistics for several types of regression models; (2) developing estimation algorithms and software for calculating the maximum likelihood estimates of the model parameters and variances; (3) finishing a reanalysis of the relationship between blood lead and blood pressure among adults in NHANES II which uses the stochastic regression model; (4) analyzing the association between lead exposures and stunting in the growth of children in NHANES II; and (5) analyzing the association between bone loss and hearing loss among the elderly in NHANES I. Over the next several years work will be completed on research papers describing the application of the stochastic regression model to the data sets from NHANES I and II.

Biometry Branch staff have been involved in the analysis of pregnancy outcomes from the Diabetes in Early Pregnancy Study. The

results of this study are described in detail in the Epidemiology Branch summary. Also, staff of the Biometry Branch have participated in a study undertaken by the Epidemiology Branch for the evaluation of the long-term effects to children exposed in infancy to chloride-deficient formula. The full description of this study is provided in the Epidemiology Branch summary.

Biometry Branch staff have also been involved with the Epidemiology Branch and Mental Retardation and Developmental Disabilities Branch, CRMC, in the planning and development of the Chorionic Villus Sampling and Amniocentesis Study. This multicenter clinical trial began its pilot phase in March, 1985. Preliminary analysis will begin soon on fetal loss rates and time to fetal death, with an appropriate application of life table techniques.

The Normal Range Study is a collaboration with the Clinical Pathology Department of the Clinical Center designed to establish reference standards for certain blood chemistries such as SED rate, hematocrit, and white blood cell counts. The Branch is currently analyzing the data provided by 1146 normal volunteers at six month intervals over 2 1/2 years.

New nonparametric techniques in exploratory data analysis are being employed in order to characterize the various distributions more completely than the usual normal theory approach would allow. Graphic methods, families of transformations, and g- and h-distributional families all are providing insight into the non-gaussian nature of these variables. This unusually complete data set will allow for analysis by covariates such as race, gender, and age as well as for estimation of the within-person variability over the 2 1/2 years of data collection. Results are currently being prepared for a series of articles characterizing the normal ranges of these measures in the medical literature.

Several developmental studies utilizing statistical time series methodology have been performed by Branch staff. These applications have been shown to be valuable for the interpretation of a diverse collection of biomedical data sets that were referred to the Branch for analysis. Digital filtering, spectral analysis, and new graphical display methods have been used to identify 20-60 second rhythms in human fetal heart rate recordings, 30-70 minute rhythms in the secretion of gonadotropins in male monkeys, and seasonal and weekly patterns in a 35-year record of oral temperature and pulse rate from one human subject. For example, in a paper recently published, several findings were reported from the analysis of ultradian rhythms in human fetal heart rate. This paper demonstrates that standard techniques of statistical time series analysis can be usefully applied to conventional recordings in order to investigate rhythms in fetal heart rate. Evidence was found for

a few specific ultradian rhythms in most of the 184 fetal heart rate recordings analyzed. In future studies, it is planned to examine ultradian rhythms in samples of normal and abnormal (complicated by diabetes or hypertension) pregnancies to determine whether these analyses could be of some clinical utility.

In order to accomodate various applications of time series analysis techniques, special methods have been developed to accommodate short ( $n < 100$ ) as well as long ( $n > 10,000$ ) multivariate time series. Simulations and Monte Carlo methods have been used to evaluate the properties of these newly-devised techniques. The data findings as well as the statistical methodology have been reported in a variety of talks and papers.

#### Other Professional Activities

Mrs. Bryla works collaboratively on research projects with staff of the Laboratory of Developmental and Molecular Immunity, IRP. Presently she is analyzing antibody titer levels on the normal adult volunteers who received pertussis toxin "toxoid". This is the first phase of the study to assess the safety, immunogenicity, duration of synthesis and protective actions of pertussis toxin "toxoid"-induced antibodies. She is also involved in the testing and evaluation of a vaccine for haemophilus influenza.

Mr. Denman serves as Adjunct Assistant Professor on the faculty of the Department of Preventive Medicine and Biometrics of the Uniform Services University of the Health Sciences in Bethesda, Maryland.

Mr. Graubard works collaboratively on several research projects with staff of the National Center for Health Statistics (NCHS). His expertise in the design and analysis of complex surveys has provided a beneficial link between our two agencies. He has also participated in the development of procedures for testing children in the NHANES III for mental retardation to be funded through an InterAgency Agreement between NCHS and NICHD.

Mr. Hoffman serves as a member of the Planning Group for the International Collaborative Effort on Perinatal and Infant Mortality (ICE), a committee sponsored by NCHS in conjunction with other U.S. Public Health Service agencies and representatives from six foreign countries. The committee is chaired by Dr. Hartford, Office of International Statistics, NCHS.

Dr. Reed assists the Better Babies Project in design aspects of studies to evaluate the effect of educational and behavioral interventions on the birth outcomes in a section of Washington, DC. Also, at the request of the NICHD Advisory Council, Dr. Reed conducted an examination of how the interview of applicants affected

priority scores assigned to proposals for Physician Scientist Awards and Clinical Investigator Awards granted by NICHD to encourage careers in clinical research. Data collected over a period of 1 1/2 years indicated that the interview slightly improved the average priority score, although this did not change the list of fundable applications.



NICHD ANNUAL REPORT  
October 1, 1986 through September 30, 1987

Biometry Branch

Publications:

Bakketeig, L.S., Bjerkedal, T., and Hoffman, H.J.: Small-for-gestational age births in successive pregnancy outcomes: Results from a longitudinal study of births in Norway. Early Human Devel. 14: 187-200, 1986.

Bercu, B., Spiliotos, B., Reed, G., and Lee, B.: Male sexual development in the monkey: IV. Further analysis of hypothalamic-pituitary-testicular function and correlation with electron and light microscopy of the testis. Journal of Pediatric Endocrinology 2: 7-22, 1987.

Damus, K., Pakter, J., Krongrad, E., Standfast, S.J., and Hoffman, H.J.: Postnatal medical and epidemiological risk factors for the Sudden Infant Death Syndrome. In Harper, R.M., and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, PMA Publishing Corporation, 1987, pp. 181-195.

Eckardt, M.J., Rawlings, R.R., Graubard B.I., Faden V.B., Martin, P.R., and Gottschalk, L.A.: Neuropsychological performance, and treatment outcome in male alcoholics. Alcoholism: Clinical and Experimental Research. (In press).

Eyster, J.T., Hoffman, H.J., DeGuire, P.J., and Denman, D.W.: Multinational comparisons of small-for-gestational age birth weight curves. ASA Social Statistics Proceedings, 1987.

Gahl, W.A., Reed, G.F., Thoene, J.G., Schulman, J.D., Rizzo, W.B., Jonas, A.J., Denman, D.W., Schlesselman, J.J., Corden, B.J., and Schneider, J.A.: Cysteamine therapy for children with nephropathic cystinosis. New England Journal of Medicine 316: 971-977, 1987.

Graubard, B.I., and Korn, E.L.: Choice of column scores for testing independence in ordered 2xk contingency tables. Biometrics 43:471-476, 1987.

Havoundjian, H., Reed, G.F., Paul, S.M., and Skolnick, P.: Protection against the lethal effects of pentobarbital in mice by a benzodiazepine receptor inverse agonist, 6,7-dimethoxy-4-ethyl-3 carbomethoxy- $\beta$ -carboline (DMCM). Journal of Clinical Investigations 79: 473-477, 1987.

Hemminki, E., Myrianthopoulos, N.C., Pomeroy, J., and Graubard, B.I.: Cesarean section as a risk factor for malformations. Int. J. Epidemiol. 15: 360-363, 1986.

Hemminki, E., McNellis, D., and Hoffman, H.J.: Patterns of prenatal care in the United States. J. Public Health Policy. (In press).

Hillman, L., Hoffman, H.J., Hasselmeyer, E.G., Jones, M., and van Belle, G.: Maternal and newborn medical risk factors for the Sudden Infant Death Syndrome. In Harper, R.M. and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, PMA Publishing Corporation, 1987, pp. 171-180.

Hoffman, H.J., Bergsjø, P., and Denman, D.W.: Trends in birth weight specific perinatal mortality: 1970-82. In Proceedings of the International Collaborative Effort on Perinatal and Infant Mortality, Volume 2. Hyattsville, Md., National Center for Health Statistics, Public Health Service, U.S. DHHS. (In press).

Hoffman, H.J., Hunter, J.C., Ellish, N., Janerich, D.T., and Goldberg, J.: Adverse reproductive factors and the Sudden Infant Death Syndrome. In Harper, R.M., and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, PMA Publishing Corporation, 1987, pp. 149-170.

Hoffman, H.J., Hunter, J.C., Damus, K., Pakter, J., Peterson, D.R., van Belle, G., and Hasselmeyer, E.G.: Diphtheria-tetanus-pertussis vaccination and sudden infant death: Results of the NICHD Cooperative Epidemiological Study of Sudden Infant Death Syndrome (SIDS) Risk Factors. Pediatrics 79: 598-611, 1987.

Hoffman, H.J., Damus, K., Krongrad, E., and Hillman, L.: Apnea, birth weight, and SIDS: Results of the NICHD Cooperative Epidemiological Study of Sudden Infant Death Syndrome (SIDS) Risk Factors (Appendix). In: Infantile Apnea and Home Monitoring (Report of the NIH Consensus Development Conference, September 29-October 1, 1986). Bethesda, Md., DHHS, NIH Publ. No. 87-2905, 1987, pp. 53-69.

Hoffman, H.J., Denman, D.W., Damus, K.H., and van Belle, G.: Comparison of matched vs. unmatched analyses in a case-control study of SIDS risk factors. ASA Social Statistics Proceedings, 1987.

Kraus, J.F., Peterson, D.R., Standfast, S.J., van Belle, G., and Hoffman, H.J.: The relationship of socioeconomic status and the Sudden Infant Death Syndrome: Confounding or effect modification? In Harper, R.M., and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, PMA Publishing Corporation, 1987, pp. 213-221.

Rawlings, R.R., Graubard, B.I., Teper, S., Ryback, R.S., and Eckardt, M.J.: Conditional quadratic discrimination in the identification of biological markers for disease screening. Biometrical Journal 28: 957-964, 1986.

Rawlings, R.R., Graubard, B.I., Faden, V.B., and Eckardt, M.J.: A study on discriminant analysis techniques applied to multivariate lognormal data. Journal of Statistical Computation and Simulation 26: 79-100, 1986.

Scheidt, P.C., Bryla, D.A., and Hoffman, H.J.: Phototherapy and patent ductus arteriosus (Letter to the Editor). Pediatrics. (In press).

Steardo, L., Marone, E., Barone, P., Denman, D.W., Moteleone, P., Cardone, G.: Prophylaxis of migraine attacks with a calcium-channel blocker: Flunarizine versus methysergide. J. Clin. Pharmacol. 26: 524-528, 1986.

van Belle, G., Hoffman, H.J., and Peterson, D.R.: Intrauterine growth retardation and the Sudden Infant Death Syndrome. In Harper, R.M., and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, PMA Publishing Corporation, 1987, pp. 196-212.

### Presentations:

Bryla, D.A.: Recollections on vaccine trials. Invited presentation for the 5th Scientific Symposium of the American Center for Chinese Medical Sciences. Falls Church, Virginia, April, 1987.

Denman, D.W.: Introduction to SASGRAPH. Invited presentation at the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences. Bethesda, Maryland, January, 1987.

Denman, D.W., Graubard, B.I., and Reed, G.F.: Smallest detectable relative risk in epidemiologic studies. Presentation for the 1987 Joint Statistical Meetings of the American Statistical Association. San Francisco, California, August, 1987.

Eyster, J.T., Hoffman, H.J., DeGuire, P.J., and Denman, D.W.: Multinational comparisons of small-for-gestational age birth weight curves. Presentation for the 1987 Joint Statistical Meetings of the American Statistical Association. San Francisco, California, August, 1987.

Graubard, B.I., Fears, T.R., Gail M.H.: Effects of cluster sampling of controls on analyses of population based case-control studies. Presentation for the 1987 Joint Statistical Meetings of the American Statistical Association. San Francisco, California, August, 1987.

Hoffman, H.J.: DTP: Results of the NICHD SIDS Cooperative Epidemiological Study. Invited presentation for the DTP Advisory Group Meeting, Boston Collaborative Drug Surveillance Program. Boston, Massachusetts, September, 1986.

Hoffman, H.J.: Federal SIDS Research Program -- Emphasizing results from the NICHD SIDS Cooperative Epidemiological Study. Invited presentation for SIDS Awareness Month in Missouri, sponsored by SIDS Resources, Inc., Old Courthouse Building. St. Louis, Missouri, October, 1986.

Hoffman, H.J.: Results of the NICHD SIDS Cooperative Epidemiological Study with respect to apnea, birth weight, gestational age at birth, and postnatal growth retardation. Invited presentation for a Conference on SIDS: Programming for the Future, sponsored by the Division of Maternal and Child Health, Health Resources and Services Administration, DHHS. Rockville, Maryland, November, 1986.



Hoffman, H.J., Damus, K.H., Hillman, L., Krongrad, E.: Risk factors for SIDS: Results of the NICHD SIDS Cooperative Epidemiological Study. Invited presentation at the International Conference on the Sudden Infant Death Syndrome: Cardio-Respiratory Mechanisms and Interventions sponsored by the New York Academy of Sciences. Villa Olmo, Lake Como, Italy, May, 1987.

Hoffman, H.J., and Damus K.H.: SIDS: Who is at risk? Fourth Annual Kevin Maloney Lecture on SIDS sponsored by the Massachusetts Center for Sudden Infant Death Syndrome at Children's Hospital. Boston, Massachusetts, June, 1987.

Hoffman, H.J.: SIDS Research Findings. Invited lecture at a meeting for Kaiser-Georgetown. Arlington, Virginia, July, 1987.

Hoffman, H.J., Denman, D.W., Damus, K.H., and van Belle, G.: Comparison of matched vs. unmatched analyses in a case-control study of SIDS risk factors. Presentation for the 1987 Joint Statistical Meetings of the American Statistical Association. San Francisco, California, August, 1987.

Korn, E. L., Graubard, B. I.: Choice of column scores for testing independence in ordered 2 x K continuing tables. Presentation for the 1987 Joint Statistical Meetings, American Statistical Association. San Francisco, California, August, 1987.

Rawlings, R. R., Graubard, B. I., Faden, V. B. and Eckardt, M. J.: A Monte-Carlo study of the effects of nonsphericity and nonnormality on repeated measures tests. Presentation for the 1987 Joint Statistical Meetings, American Statistical Association. San Francisco, California, August, 1987.

Reed, G.F.: The exact distribution of the dispersion test for randomness of binary sequences. Presentation for the 1987 Joint Statistical Meetings of the American Statistical Association. San Francisco, California, August, 1987.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00801-12 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies Based on the Medical Birth Registries of Norway and Sweden

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB PRP NICHD

Other: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) Inst. of Hygiene & Soc. Med. & Dept. of OB/GYN, Univ. of Bergen, Norway (P. Bergsjø and L. Irgens); Dept. of Community Medicine, Univ. of Trondheim and Nat'l Inst. of Public Health, Oslo, Norway (L. Bakketeig, A. Arntzen); Dept. of OB/GYN and Social Med., Uppsala Univ. (G. Lindmark, S. Cnagtingius, O. Meirik).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.4

## PROFESSIONAL:

.2

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway and Sweden, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, (3) perinatal mortality in relation to order of birth and size of sibship, (4) epidemiologic risk factors for preterm birth, and (5) epidemiologic risk factors for small-for-gestational age births.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00802-12 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB PRP NICHD

Others: Daniel W. Denman Mathematical Statistician BB PRP NICHD  
Geoffrey D. Birky Math. Stat. (Summer) BB PRP NICHD

COOPERATING UNITS (if any) PRP, NICHD (H.W. Berendes, M.D. Overpeck); CRMC, NICHD (A. Willoughby); EB, BRAP, NIEHS (A.J. Wilcox); Departments of Health in the following states: Michigan, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, NCHS (R. Hartford).

LAB/BRANCH  
Biometry Branch

## SECTION

INSTITUTE AND LOCATION  
NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.4

## PROFESSIONAL:

.2

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

Several research contracts have been jointly funded by NICHD and NIEHS to provide data from selected U.S. States (listed above) to compare with data from other developed countries (Australia, Japan, Norway and Scotland) for the time period, 1980-84. This study is entitled: Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00803-03 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB PRP NICHD

Others: Karla H. Damus Consultant BB PRP NICHD  
Jehu C. Hunter Consultant BB PRP NICHD  
Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) U. Wash., (D. Peterson; G. van Belle); Loyola U. (J. Goldberg); UCLA (R. Harper and J. Kraus); Columbia U. (J. Pakter, E. Krongrad); N.Y. State Health Dept. (S. Standfast); U. Mo. (L. Hillman); U. London, U.K. (D. Southall); U. Miami (M. Dapena); U. NM (P. McFeeley); AFIP, Washington, D.C. (T. Stocker).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.4

## PROFESSIONAL:

.3

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The NICHD Cooperative SIDS Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 838 SIDS cases (800 singleton and 38 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of neuro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons will be made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00811-08 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

National Collaborative Cysteamine Study Data Center

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: George F. Reed Mathematical Statistician BB PRP NICHD

Other: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) Computer Sciences, PRP, NICHD (E. Harley and E. Nelson); HGB, IRP, NICHD (W. Gahl); Univ. California, San Diego (J. Schneider); Uniform Services Univ. of the Health Sciences (J. Schlesselman); Univ. of Michigan Medical School (J. Thoene).

LAB/BRANCH  
Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.1

## PROFESSIONAL:

.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This study was a clinical trial to determine the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis, a rare inborn metabolic disease which usually leads to end-stage renal disease before 10 years of age. All children enrolled in the trial received oral cysteamine. Control information was provided by data collected on 64 patients who participated in a previous trial evaluating ascorbic acid for the treatment of this disease. The cysteamine trial enrolled 94 patients; analysis of data is completed, and major findings have been published.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00813-06 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biostatistical Methods for the Analysis of Laboratory Research Studies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George F. Reed Mathematical Statistician BB PRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB PRP NICHD  
Barry Graubard Mathematical Statistician BB PRP NICHD  
Howard J. Hoffman Chief BB PRP NICHD  
Geoffrey Birky Math. Stat. (Summer) BB PRP NICHD

## COOPERATING UNITS (if any)

NS, LBC, NIADDK (P. Skolnick); CPD, CC, NICHD (R. Elin and M. Ruddlell); IRP, NIAID (D. Alling); Dept. of Statistics, Harvard U. (D. Hoaglin).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.2

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research in design and analysis problems arising from laboratory studies on:  
(1) dose-response relationships, (2) bioassay and potency estimation,  
(3) time to event, life table analyses, and (4) other investigations of the effects of external stimuli.

In addition to work on techniques for estimating tolerance limits for chemical residue depletion in animals, which has been submitted for publication, a major effort in this research area has arisen in the analysis of data from the Clinical Center's Normal Range Study. This study has resulted in the collection of a large number of biochemical and clinical measurements taken serially for 2 1/2 years from "normal" volunteers. The object of the analysis is to characterize the distribution of each variable in order to determine values that can be considered normal. Some of the statistical techniques to be applied will be exploratory data analysis methods, including graphical techniques and outlier detection, transformation of variables, analysis of variance components, and serial correlation. The results of this project will appear in several published reports of quantitative characterizations with special reference to factors that may affect these distributions, such as smoking, drinking, and eating habits, and other demographic or socio-economic factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00818-06 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Research in Developing Nonparametric Methods for Biomedical Applications

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: George F. Reed Mathematical Statistician BB PRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB PRP NICHD  
Howard J. Hoffman Chief BB PRP NICHD

COOPERATING UNITS (if any)

LCDB, NIDDK (L. Amende and J. Blanchette-Mackie).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS

.2

PROFESSIONAL:

.2

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The objective is to investigate and develop distribution-free methods in areas of application for which standard parametric techniques are inappropriate or too sensitive to violations of underlying assumptions.

Much of the work of the Branch lends itself to the nonparametric approach. In sample size studies involving analysis of 2x2 tables, the determination of the minimum detectable risk for a given sample size is often required. Some asymptotic techniques have been developed in the Branch for this, but they must ultimately be validated by an exact technique which is theoretically based on the theory of randomization testing. This technique is now being developed. Another general application is the use of runs tests to evaluate residuals in regression analysis to determine goodness of fit. Research on a particularly apt nonparametric runs test, based on the variance of the length of positive and negative runs of residuals, continues. Investigation is also continuing in the use of randomization testing for comparing proportions with cluster effects.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00820-06 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methods for Epidemiologic Data

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

Others: Barry I. Graubard Mathematical Statistician BB PRP NICHD  
Howard J. Hoffman Chief BB PRP NICHD  
George F. Reed Mathematical Statistician BB PRP NICHD

## COOPERATING UNITS (if any)

Biomathematics Department, School of Medicine, UCLA (E. Korn).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.3

## PROFESSIONAL:

.3

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since many epidemiologic problems cannot be solved by standard techniques, new methods can help extract more complete answers from research data. The objective of this project is to use mathematical theory and computer simulations to develop and evaluate statistical methods appropriate to data arising in epidemiologic research, and to carry out the statistical programming needed to make these methods easily available to other researchers. This may include evaluating outside computer software, using standard programs in novel ways, and writing special purpose programs.

Further study will continue in the use of generalized linear models and the SAS procedure GLM in regression, analysis of variance, and analysis of covariance. Methods appropriate to categorical data and contingency tables will also be given special attention. Useful techniques will be presented in seminars and publications in statistical journals.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00821-05 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of New Graphical Methods for the Analysis of Biomedical Data

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

Others: Howard J. Hoffman Chief BB PRP NICHD  
George F. Reed Mathematical Statistician BB PRP NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.2

## PROFESSIONAL:

.2

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Statistical graphics are an integral part of the analysis and presentation of data. Rapid development in this field is evidenced by an extensive research literature and a host of new computer graphics technologies.

The object of this project is to draw from current literature and computer demonstrations and develop graphical methods for: (1) more effective statistical analysis, particularly of multi-dimensional data sets and time-dependent variables; and (2) for more easily understood summaries in finished presentations.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00840-06 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Statistical Discriminant Methods with Applications to Alcoholism Screening

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

## COOPERATING UNITS (if any)

Alcohol, Drug Abuse and Mental Health Administration (R. Rawlings, S. Teper, V. Fadden and M.J. Eckardt).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.05

## PROFESSIONAL:

.05

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study investigates the statistical properties of a variety of discriminant functions and determines how well they differentiate between alcoholic, other diseased, and normal populations using standard batteries of blood chemistries. Blood chemistry variables that are used to discriminate between diseased and normal groups have been found to have skewed distributions. Using computer simulations, the properties of parametric (linear and quadratic) and nonparametric (fixed and variable kernel) discriminant methods have been investigated when the data comes from a skewed multivariate lognormal distribution. In addition, rank and inverse normal score transformations were applied to the data from the simulation in order to determine if they could improve upon the accuracy of the discriminant functions. It was found that the nonparametric methods were less accurate than the parametric methods when the data came from a multivariate lognormal distribution. The rank and inverse normal score transformations greatly improved the classification accuracy of the parametric methods.

The rank and inverse normal score transformations have been applied to data from multivariate repeated measure designs in order to remedy the effect nonsphericity and non-normality has upon classical repeated measure analyses. It was shown through simulations that the inverse normal scores does improve the performance of certain classical tests used with repeated measures.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00841-06 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methods for Comparing and Analyzing Data from Several Complex Surveys

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

Other: Howard J. Hoffman Chief BB PRP NICHD

## COOPERATING UNITS (if any)

EDB, NIA (D. Brock); Research Triangle Institute (B.V. Shah).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.2

## PROFESSIONAL:

.2

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will develop statistical methods for the analysis of data from complex designed surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). Existing multiple linear regression methods for the analysis of data from complex surveys are compared to newly developed regression methods. These regression methods will be applied to the NHANES data sets to determine if they can be used to provide new information on the complex relationships of growth and nutrition. The preliminary results from this research indicate that the newly developed regression models can better describe complex relationships in the data. This research is being pursued in part through a research contract with the Research Triangle Institute to work in collaboration with NICHD to carry out this study. Over the course of this contract, manuscripts will be prepared for publication which will present the results of the study along with the development of computer programs for applying the methods to real data.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00842-05 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Development of Statistical Methods to Analyze Cluster Samples

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

Other: Howard J. Hoffman Chief BB PRP NICHD

## COOPERATING UNITS (if any)

BB, PRP, EMS, NCI (M. Gail and T. Fears).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.2

## PROFESSIONAL:

.2

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

This research project will study statistical methods for analyzing categorical data that comes from cluster samples where the observations within each cluster may be correlated and where the observations may be selected with unequal probabilities. In particular, the analysis of cluster samples from population-based case-control studies and cross-sectional and longitudinal health surveys is examined. Research has concentrated on developing modifications to logistic regression and Mantel-Haenzel and Wolf-Haldane procedures that would account for the complex sample design. Computer simulations are used to validate statistical approximations used in the development of modified methods. Preliminary results from this research indicate that the modified methods for analyzing data from cluster samples appropriately take into account the intra-cluster correlation structure and the unequal weighting of the observations. These methods will be useful for analyzing infant feeding studies and repeat pregnancy studies where the family constitutes the cluster.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00843-04 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An Investigation of Matched Analysis in Case-Control and Cohort Studies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

Others: Howard J. Hoffman Chief BB PRP NICHD  
George F. Reed Mathematical Statistician BB PRP NICHD

## COOPERATING UNITS (if any)

Biostatistics Department, School of Medicine, UCLA (E. Korn).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.05

## PROFESSIONAL:

.05

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will investigate the validity and efficiency of neighborhood matching for case-control and cohort studies. The National Health and Examination Surveys I and II data were used in conjunction with neighborhood codes (i.e., specifying which individuals in the sample lived close together) to empirically determine the effect neighborhood matching would have upon validity and variance of estimates of risk of various conditions with respect to differing exposures. It was demonstrated that for some types of exposure-condition relationships, neighborhood matching was useful for controlling for confounding. However, there was a loss in efficiency due to a reduced number of matchable observations and a smaller number of degrees of freedom in the test statistics. These empirical examples can provide some guidance to researchers who contemplate neighborhood matching for an observational study. This project is one of the first known attempts of investigating the effect neighborhood matching has upon the analysis of observational data.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00850-11 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB PRP NICHD

Others: Howard J. Hoffman Chief BB PRP NICHD  
Barry I. Graubard Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) Office of the Associate Director, PRP, NICHD (H. Berendes); Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson); Computing Sciences Consultant (K. Fetterly).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.6

## PROFESSIONAL:

.4

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study, which began in 1974, is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants were followed through six years of age.

The Biometry Branch served as a data center for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency. The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. The results of the newborn data were published in a supplement to Pediatrics in February 1985. It is anticipated that manuscripts on the follow-up data will be submitted for publication by the end of 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00852-05 BB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1980 National Natality Survey and Fetal Mortality Survey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB PRP NICHD

Others: Howard J. Hoffman Chief BB PRP NICHD  
Barry I. Graubard Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any)

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); National Center for Health Statistics, Division of Vital Statistics, Natality Statistics Branch (P. Placek).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:

.1

PROFESSIONAL:

.1

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The 1980 National Natality Survey and 1980 National Fetal Mortality Survey conducted by the National Center of Health Statistics (NCHS) contains data on 9,941 live births and 6,386 fetal deaths. For each live birth and fetal death certificate selected, mother, physician, hospital and radiation questionnaires were obtained by NCHS. This project has provided data on a nationwide sample relating to pregnant women's characteristics, outcome of pregnancy, labor and delivery.

During this year, planning meetings for the proposed 1988 National Maternal and Infant Health Survey (NIMIHS) were held with the National Center of Health Statistics (NCHS). It is proposed that information will be collected for three national samples of vital records: 10,000 certificates of live births, 6,000 reports of fetal deaths, and 4,000 death certificates for infants. Based on the earlier collaboration with the Biometry Branch for the 1980 surveys, NCHS staff has worked closely with us in formulating the proposed content of questionnaires for NIMIHS.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00853-03 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB PRP NICHD

Other: George F. Reed Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) Office of the Director, NICHD (C. Lowe); Laboratory of Developmental &amp; Molecular Immunity, NICHD (J. Robbins); TEKU Hospital, Nepal (I. Acharya).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.5

## PROFESSIONAL:

.3

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is a cooperative, randomized trial to determine the efficacy of Vi polysaccharide in preventing typhoid fever in Nepal. The Biometry Branch's involvement in this study is to design data collection forms, and assist in the data management and the analysis with the study investigators from NICHD and Nepal.

In March 1986, 6,912 volunteers from five villages in Nepal were randomly vaccinated with either the Vi polysaccharide or pneumococcal vaccine. These volunteers will be visited every three days for the next two years to verify their health status and to detect any typhoid cases prior to treatment. Blood cultures will be done on anyone with a fever of three days duration. The results of the randomization will not be available until late in 1988.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00854-03 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Analysis of MCH Data from the National Longitudinal Youth Survey

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB PRP NICHD

Other: Howard J. Hoffman Chief BB PRP NICHD

COOPERATING UNITS (if any) Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis);  
Ohio State University (F. Mott).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.1

## PROFESSIONAL:

.1

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has as its primary objective to analyze and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. In addition, a wealth of other data have been collected on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The collection of data on pregnancy outcome and the first year of life of the child began in 1983 and ended in 1986. With this four year data base, analysis of trends over time in the maternal and child health can be done.

The Biometry Branch has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00860-07 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Biomedical Time Series Data

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB PRP NICHD

Other: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) CI, CP, GRC, NIA (M. Brock); Dept. of Pediatrics, Univ. of South Florida College of Medicine, St. Petersburg, Florida (B. Bercu); Pediatric Nutrition, Mead Johnson Company (J. Hansen); Dept. of Obstetrics & Gynecology, Univ. of Cambridge, England (K. Dalton and G. Breborowicz).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.4

## PROFESSIONAL:

.2

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are: (1) to characterize developmental patterns from daily measurements of gonadotropins and for estrogens in premenarchial girls and pubescent boys based on radioimmunoassay methods for measuring urinary luteinizing hormone, urinary follicle stimulating hormone, and urinary estradiol, estriol and estrone hormones; (2) gonadotropins in both castrated and intact male monkeys of different ages; (3) growth hormone in normal and precocious pubertal children; (4) to assess circadian and other rhythms in heart rate, temperature and other serial data collected from long-term studies in humans; and (5) to perform analysis of these serial measurements using methods of statistical time series analysis, including autoregressive filtering, auto- and cross-spectrum analysis, and robust smoothing procedures.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00861-05 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB PRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) PRP, NICHD (H. Berendes); CRMC, NICHD (D. McNellis); Univ. of Trondheim, Norway (G. Jacobsen, L. Bakketeig); U. of Bergen, Norway (P. Bergsjø, T. Evans, T. Markestad); Uppsala Univ., Sweden (G. Lindmark); Bell Communications, Livingston, N.J. (G.W. Reed); U. of Alabama in Birmingham (R. Goldenberg).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.4

## PROFESSIONAL:

.3

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala). The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00870-04 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Long-Term Reproductive Effects of Cesarean Section Birth

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

Other: Howard J. Hoffman Chief BB PRP NICHD

COOPERATING UNITS (if any) DNB, CDNDP, NINCDS (N. Myrianthopoulos); University of Helsinki, Department of Public Health, Finland (K.E. Hemminki); New York State Department of Health (D. Glebatis, D. Janerich and G. Therriault); National Center for Health Statistics, Family Growth Branch (W. Mosher).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.05

## PROFESSIONAL:

.05

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the work is to study long-term adverse effects possibly following a delivery with cesarean section. Effects on subsequent fertility, ectopic pregnancies and on malformations of subsequent children having been studied using U.S. data. Subsequent fertility is studied by comparing women having had a cesarean section to those having had a vaginal delivery in their first pregnancy using data from the 1982 National Survey of Family Growth. Effect on ectopic pregnancies is studied by comparing the past delivery history of women having had ectopic pregnancy to that of women having had a live birth or a spontaneous abortion. The data source is fetal and live birth certificates in Upstate New York. Effects on malformations are studied by comparing the malformation rates of children whose mothers have had a previous cesarean section to that of children whose mothers have had a previous vaginal delivery. The data source is the Collaborative Perinatal Project. Many different types of problems, both for the mother and infant, in the subsequent pregnancies have been studied using the data in the Swedish Birth Register. Subsequent studies include linking this data to the hospital discharge register to study problems not related to pregnancies ending in birth.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00871-02 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Trial of New Drug Therapy for Cystinosis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George F. Reed Mathematical Statistician BB PRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB PRP NICHD

COOPERATING UNITS (if any) HGB, IRP, NICHD (W. Gahl); Univ. California, San Diego (J. Schneider); Univ. of Michigan Medical School (J. Thoene); Univ. of Texas Health Science Center, Dallas (J. Reisch).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.3

## PROFESSIONAL:

.3

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Cysteamine Study provided answers to the question of the drug's efficacy with some inferential difficulty, since cysteamine's unpleasant taste and smell rendered it unpalatable to many patients, who subsequently did not receive effective amounts of the drug. The design of the study itself, with no randomized concurrent control group, obscured effects and required a good deal of reliance on adjustment techniques in the final analysis.

There exists a chemical analog to cysteamine, phosphocysteamine, which is more palatable and demonstrates cystine depleting properties, although it has not been subjected to a rigorous clinical test of efficacy. The object of the study is to compare treatment with phosphocysteamine to cysteamine therapy in a randomized clinical trial. If some other drug with therapeutic promise is made available early enough in the study, then it, too, may be included in trial.

Patient recruitment and treatment will be coordinated at a contracted study center at the University of California, San Diego. Data center functions will be performed at the University of Texas Health Science Center at Dallas. The study will encompass 3-4 years of enrollment and treatment of at least 80 patients. The drug will be evaluated on the basis of renal function as measured by serum creatinine levels and creatinine clearance, as a surrogate of glomerular filtration rate, at the end of the study.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00872-02 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Associated with Premature Births: Missouri Follow-back Survey

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB PRP NICHD

Others: Howard J. Hoffman Chief BB PRP NICHD  
Karla Damus Consultant BB PRP NICHDCOOPERATING UNITS (if any) Pregnancy and Perinatology Branch, CRMC, NICHD (A. Willoughby);  
Missouri Division of Health (G. Land, W. Schramm, and J. Stockbauer).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.1

## PROFESSIONAL:

.1

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, for calendar year 1987 than is now available from the United States vital records. This objective will be accomplished by the following: (1) to design and administer a mail questionnaire to mothers of VLBW infants, mothers of all fetal deaths, and a sample of mothers of LBW infants (1,500-2,499 grams) and normal birth weight infants (>2,500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; (2) to design and conduct telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on mail questionnaires; (3) to develop and conduct procedures for ascertaining from hospital and physician records unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and (4) to prepare and deliver an edited data tape to NICHD. In addition, mortality will be ascertained throughout the first year of life for this birth cohort. This information will help to answer the question: Has there been a reduction in neonatal mortality at the expense of an increase in post-neonatal mortality for these infants?

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00873-01 BB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Relationship of Mother's Prepregnancy Size to Pregnancy Complications and Outcome

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

Other: Howard J. Hoffman Chief BB PRP NICHD

COOPERATING UNITS (if any) EB, PRP, NICHD (J. Mills).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.1

## PROFESSIONAL:

.1

## OTHER

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This project will study the relationships between the prepregnant body mass size of a woman and the risk of adverse pregnancy complications and pregnancy outcomes. The Kaiser-Permenante Walnut Creek malformation data set will be used for the analysis. The results from this study could help obstetricians to inform prospective mothers about the potential dangers that obesity and underweight can have upon their fetuses.





## EPIDEMIOLOGY BRANCH

- Z01 HD 00318-07 A Prospective Study of the Frequency and Duration of Infant Feeding Practices  
N. Kurinij
- Z01 HD 00323-07 District of Columbia Perinatal Study  
H. W. Berendes
- Z01 HD 00325-06 Neural Tube Defects and Folate  
J. L. Mills
- Z01 HD 00329-05 Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.  
H. W. Berendes
- Z01 HD 00331-04 Diabetes in Early Pregnancy Project (DIEP)  
J. L. Mills
- Z01 HD 00332-04 The Risk of Adverse Pregnancy Outcome Following Cervicitis During Pregnancy  
R. P. Nugent
- Z01 HD 00333-04 Congenital Anomalies and In Vitro Fertilization (IVF)  
J. L. Mills
- Z01 HD 00334-04 Low Birth Weight Across Generations  
M. A. Klebanoff
- Z01 HD 00340-04 Ethnic Differences in Birth Weight and Length of Gestation  
P. H. Shiono
- Z01 HD 00341-04 Cesarean Childbirth Rates in the U.S.  
P. H. Shiono
- Z01 HD 00343-04 The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins  
H. W. Berendes
- Z01 HD 00344-04 Long Term Effects of Infant Formulas Deficient in Chloride  
M. H. Malloy
- Z01 HD 00346-03 Time Trends in the Incidence of Biliary Atresia  
M. A. Klebanoff
- Z01 HD 00352-02 Studies of Human Immunodeficiency Virus - Related Problems  
G. G. Rhoads
- Z01 HD 00360-01 A Prospective Study of 1st Trimester Use of Bendectin and Malformations  
P. H. Shiono

- Z01 HD 00361-01 Child Health Supplement to the 1988 National Health  
Interview Survey  
M. D. Overpeck
- Z01 HD 00362-01 Nutritional Aspects of Perinatal Epidemiology  
in Central America  
J. Villar
- Z01 HD 00832-04 Changes in Perinatal and Infant Mortality by Race in  
Selected U.S. Cities  
L. C. Cooper and M. D. Overpeck

NICHD Annual Report  
October 1, 1986 through September 30, 1987

Epidemiology Branch, Prevention Research Program

The Epidemiology Branch has continued to be involved in a wide variety of projects relating to maternal and child health. For purposes of this report Branch activities are described in five categories: 1) Low Birth Weight and Perinatal Mortality; 2) Teratologic and Genetic Problems; 3) Human Immunodeficiency Virus; 4) Nutrition; and 5) Other.

Low Birth Weight and Perinatal Mortality

The reasons for the large ethnic differences in the incidence of low birth weight and preterm delivery are unknown. Known risk factors such as smoking, level of maternal education, restricted maternal weight gain, and a variety of obstetrical conditions do not explain the two-fold increase in incidence in low birth weight among Black women as compared to White women in the U.S. Nor do they explain why Hispanic women, despite their relatively low economic status and lack of formal education have relatively low rates of low birth weight. The Branch is currently working to find previously undescribed reasons for these discrepancies. At prenatal clinics affiliated with Columbia and Northwestern Universities, data will be prospectively obtained from pregnant women from five ethnic groups: American Black, Chinese, Mexican, Puerto Rican, and White. This information will include such topics as social support, level of physical activity, nutrition, stress, beliefs and attitudes about pregnancy, and acculturation. The study instruments for this study have been completed and piloting of the instrument and formal data collection will begin in the fall of 1987.

Data analysis has begun this year on a case-control study of low birth weight in the predominantly black population in the District of Columbia. Low birth weight cases occurring in six hospitals accounting for about 85% of such births to District residents were interviewed. Control women with normal birth weight babies were also studied. A variety of demographic, lifestyle and medical factors are being examined to identify particular characteristics in this population which might predispose to low birth weight.

In another inner city initiative, the Branch has collaborated with several private sector organizations in the Better Babies Project. The project is aimed at reducing the rate of low birth weight infants in a target area in the District of Columbia. Outreach workers are identifying as many pregnant women as possible in a specific target area of the District and encouraging them to begin

prenatal medical care, improve the frequency and total number of their prenatal visits, improve their adherence to health and medical advice and link them with specific interventions designed to reduce prematurity, smoking, and social stress. Branch staff have provided recommendations on study design and types of intervention and will be responsible for evaluating the impact of the project on low birth weight. An extensive pilot project was completed on August 31, 1986. The formal trial began September 1, 1986 and is expected to continue through 1990.

Washington, DC and certain other cities have been cited for unusually high rates of infant and perinatal mortality. However, concern with reliability of reporting of early fetal deaths and ambiguous classification between live births and fetal deaths has led to uncertainty in comparing fetal and infant deaths among various jurisdictions. To address this problem Branch staff have developed special methods to describe perinatal mortality for 59 U.S. cities over the period 1972-1981. These data will allow analysis based on losses after 24 or 28 weeks gestation, which is more reliable than the usual data on fetal deaths which are based on 20 weeks gestation. By combining late fetal and neonatal deaths, reasonably comparable perinatal mortality statistics can be shown among the 59 jurisdictions. Examination of shifts from neonatal to post-neonatal mortality and correlation with regionalization of care and size of city have been made.

It is known that low birth weight tends to recur across generations. Evidence from the linked birth certificates of mothers and children born in Tennessee indicates that the rate of intrauterine growth mediates this effect more strongly than does length of gestation. For example, mothers who weighed 2000-2499 grams at birth are nearly 4 times as likely to have a small for gestational age infant compared to mothers who weighed 4000-4499 grams, but only 1.6 times as likely to give birth to a preterm infant. It was not possible to evaluate the effect of the mother's own gestational age at birth. In order to determine which of these mechanisms is operating, it will be necessary to acquire data sources from the early 1960's in which both length of gestation, birth weight, and other confounding factors were recorded for subjects who can be traced and whose own reproductive performance can be assessed at the present time. Data of this type are being assembled from a health district in Sweden which maintained a low birth weight registry in the 1950's. Two contracts were initiated this year to study the intergenerational associations of birth weight, gestational age, and possibly other perinatal complications. One contract with the University of Pennsylvania and Brown University will trace girls who were members of the Philadelphia and Providence cohorts of the Collaborative Perinatal Project (1959-66). The other contract with the University of Southern California and the Psykologisk Institut in Copenhagen



will locate girls who were subjects in the Danish Perinatal Study (1959-61). In each study all girls who were born preterm or small for gestational age, and a random sample of controls will be located and their reproductive outcomes determined. Subjects to be traced and data collection instruments are currently under development.

The role of genital tract infection in the causation of low birth weight is under continuing exploration. Pathologically defined chorioamnionitis is known to be much more common in preterm than in term births, but the literature relating carriage of particular vaginal or cervical organisms to the onset of labor has been confusing. A major project to examine these issues, funded by CRMC and NIAID, is being largely coordinated by Branch staff. More than 9000 women have been enrolled in five medical centers across the country with eventual enrollment projected to be between 12,000 and 18,000. Vaginal and cervical cultures are being performed on participants during the second trimester of pregnancy. A variety of organisms are being sought. Outcomes are being monitored in terms of subsequent complications of pregnancy, intrapartum events, and perinatal outcome. Women carrying Group B streptococci, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* are being invited to participate in a randomized trial of long term erythromycin therapy (1 gram daily) in order to assess its prophylactic effect. To date over 1800 women have agreed to be randomized including 1581 with *Ureaplasma*, 467 with Group B streptococcus and 203 with *Chlamydia*. (Some women have more than one organism.)

In a related but smaller project approximately 800 women attending the Johns Hopkins University prenatal clinic have been enrolled in a study including careful observation and photographs of the cervix in the second trimester of pregnancy. Cultures for multiple organisms were also taken. Follow-up of the women has been completed and the data analysis has begun. Results so far suggest that cervical inflammation is difficult to define in a reproducible way, which is likely to make it difficult to use the concept clinically. Within this inner city population *Chlamydia* colonization was more common in Black (15.4%) than in other (6.9%) women. A paper presenting the major findings of the study has been submitted for publication. *Mycoplasma hominis*, *Chlamydia trachomatis*, heavy smoking and delivery of a previous low birth weight infant were associated with preterm birth. *Chlamydia*, *Candida albicans*, maternal smoking and drinking were associated with intrauterine growth retardation.

## Teratologic and Genetic Problems

The Branch has continued its work on a number of projects relating to the etiology and prevention of congenital malformations. A variety of malformations are more common in births to diabetic women and it is clear that these are determined (based on their embryology) in the first 6 weeks after conception. The Diabetes in Early Pregnancy Project (DIEP) has recruited women before or within 21 days of conception to identify early pregnancy in 422 diabetic pregnancies and 494 control women. Upon confirmation of pregnancy, the status of the diabetic women was assessed and they were taught to monitor their blood glucose levels at home on a daily basis. Blood was collected on a weekly basis through the first 12 weeks of pregnancy so that metabolic control was closely monitored.

Initial analyses from the DIEP have now been completed. The first important results from the study have come from the fetal ultrasound findings. It was determined that women in both the diabetic and control groups who had a normal fetus on 8 week ultrasound had an extremely small chance of subsequent miscarriage. This is an important finding because many clinically recognized spontaneous abortions occur after the 8th week and many investigators had assumed that these fetuses were alive until just before clinical abortion occurred. The major DIEP analysis of congenital malformations in relation to diabetes has been completed and is being written up for submission for publication. Two important and statistically significant findings appeared in this study. First, diabetic women who registered after the 21 day cut-off for full participation in the DIEP had significantly higher malformation rates in their offspring (9%) than diabetic women who entered early (4.9%). Second, diabetic women entering early still had persistently higher malformation rates than normal control subjects (4.9% vs. 2.1%). The second stage of this analysis examined the relationship between glucose control and the risk for congenital malformations. Extensive examination of glycosylated hemoglobin values and home glucose monitoring results revealed no relationship between the level of glucose control during organogenesis and the risk for congenital malformations. This result appears in sharp contrast to previous reports suggesting that women with good glycemic control were at a reduced risk for congenital malformations. A number of other DIEP analyses are now underway: 1) The relationship between intrauterine growth retardation on ultrasound and the risk for congenital malformations and the risk for intrauterine growth retardation in the diabetic versus the normal population, 2) the relationship between metabolic control of diabetes and fetal size, 3) changes in diabetic control as reflected in glycosylated hemoglobin levels during pregnancy, 4) the relationship between maternal diabetic control during pregnancy and infant size (macrosomia). Analyses in the planning stage include: 1) rates

of fetal loss in diabetic and normal pregnancy, 2) the risk of fetal loss in relation to maternal metabolic control, 3) genetic factors in diabetes associated malformations and fetal losses, 4) possible patterns of minor congenital anomalies that are characteristic of infants of diabetic mothers, 5) associated endocrine diseases as possible markers for bad obstetrical outcome in diabetic women, and 6) the descriptive epidemiology of risk factors for early fetal loss.

It has long been known that the incidence of neural tube defects (NTD) is subject to some environmental influence which must account for the variation in frequency of this malformation over time and between populations. Recent reports from Great Britain have suggested that periconceptional vitamin supplementation may prevent NTD and have implicated folate more specifically as the active ingredient. The Branch is conducting a case-control study in Illinois and California in cooperation with Northwestern University and the California State Department of Health. We are recruiting neural tube defect cases as well as two groups of control women: those having an ostensibly normal pregnancy as well as a group having a fetus or child with a major medical problem. Cases and controls are being interviewed (by telephone) about three months after birth (or prenatal diagnosis) with special reference to their use of supplementary vitamins around the time of conception. To date we have interviewed over 300 triplets (case, abnormal control, normal control) in California and 100 triplets in Illinois. Our last cases will be identified in July of 1987 and our last control subjects in October of 1987. At that point we will begin the data editing and the analysis phase of the study. The total number of subjects available for analysis is already adequate. Case ascertainment, interviewing and data collection at the data center have been progressing well.

A second study of the relationship between vitamins and neural tube defects is currently in the planning stage. Branch members have been working with investigators at the Finnish National Health Institute to use the Finnish Birth Defects Registry and Finnish blood storage facility. We hope to identify women who delivered an infant with a neural tube defect between 1982 and 1987 along with suitable control subjects. We will then go to the stored blood specimens drawn early in these pregnancies. These specimens will be collected and shipped to Dr. Neville Coleman's laboratory in New York City. He will assay folate and vitamin B12 levels in all of them without knowledge of the subject's status. We will then analyze the results to determine whether there is an association between low folate or vitamin B12 levels in early pregnancy and a risk for neural tube defect child.



Data gathering for our study of congenital malformations and in vitro fertilization is now virtually complete. Participation rates were very high in both the IVF and control groups and we estimate that we will have 80 subjects in each group available for analysis. Because the number of subjects available is modest an extensive examination was used to identify all malformations, including ultrasonography and echocardiography.

The Branch has continued its involvement in coordinating the NICHD Chorionic Villus Sampling (CVS) Study. CVS is done between 8 and 12 weeks after the last menstrual period and provides prenatal diagnosis 1-2 months earlier than does amniocentesis. The accuracy of the procedure will be assessed in all consenting patients having CVS at one of the seven participating centers. Those at average obstetric risk who live within 1-2 hours driving distance of the centers and who have a baseline ultrasound showing a viable pregnancy of 49-90 days gestational age will be used to assess the safety of the procedure. A considerable effort is being expended at each center to recruit control women who inquire about prenatal diagnosis early enough for CVS but who elect to have amniocentesis instead. As of April 24, 1987, 3362 women have been recruited into the Safety Study (2556 CVS, 806 amniocentesis) and an additional 2214 women have been entered into the CVS accuracy study. The total loss rate in women intending to continue their pregnancies after CVS has been estimated in 1700 women who were due to deliver by December 31, 1986 and was just over 4%. There were losses before 16 weeks in 2.6%, losses after 16 weeks in 1.4%, and 0.1% stillbirths. An additional 0.2% died in the neonatal period.

A transabdominal approach to CVS is being used in an increasing number of prenatal diagnosis centers and the feasibility of randomizing patients between the transcervical and transabdominal approaches is being explored in the context of the NICHD collaborative study.

#### Human Immunodeficiency Virus in Mothers and Children

Collaborative studies of vertical transmission of HIV infection in Haitian and drug-addicted women in New York have continued this year. Eighty-nine women have been enrolled in the study. Sixty-five infants have been born, 44 to drug using mothers and 21 to Haitian mothers. A total of 27 of these women (of both groups) were seropositive for HIV. The mean duration of pediatric follow-up is 5.5 months (range 0-12 months). Two infants have AIDS, 3 have persistent hepatosplenomegaly and lymphadenopathy and 4 have persistently palpable lymph nodes in multiple anatomical sites.

Progress is being made on a protocol for the study of HIV infection in hemophiliac children. It is anticipated that the study will be



funded through HRSA and will enroll HIV positive and HIV negative children as well as some sibling controls of the positives. The data center for this study will be funded by contract directly from PRP.

A study of intravenous immunoglobulin (IVIG) in the amelioration and prevention of disease in HIV infected children is also being initiated in collaboration with CRMC and NIAID. It is anticipated that this will be a randomized study with enrollment beginning toward the end of fiscal 1987. It is hoped that approximately 340 children will be enrolled some of whom will be symptomatic and some pre-symptomatic. A data center will be recruited to assist with this study and will be supervised by PRP staff.

### Nutrition

The Branch has continued to be involved in several projects relating to nutrition during pregnancy and childhood. A study has been carried out to investigate the underlying reasons for differences in breast-feeding rates between white and black women. Primiparae (n=1179) were interviewed during the first few days postpartum to ascertain their infant-feeding behavior and the factors which led them to choose exclusive breast feeding, breast and formula feeding, or formula feeding. These women were followed through the first year with a series of interviews to ascertain when they actually stopped breast feeding and their reasons for stopping. Ethnic differences in the rate of breast feeding are evident with 84% of white women breast feeding at birth compared to only 49% of black women giving birth in the three hospitals selected for study. The influence of sociodemographic factors on the incidence and duration of breast feeding was examined. Maternal educational level was strongly associated with breast feeding, whereas the effect of ethnicity was moderate. Women with some college or some graduate school education had adjusted odds of breast feeding that were 2.6 (95% C.L., 1.9-3.7) and 5.2 (95% C.L., 2.7-10.2) times higher than women with a high school education or less. In contrast, the adjusted odds of breast feeding were 2.0 (95% C.L., 1.4-3.1) times higher for white women compared to black women. The odds of breast feeding increased among black women if they attended childbirth classes, were married, or were older. Among black women the frequency of breast feeding dropped sharply by one-month postpartum. Breast-feeding duration for black vs. white women was 74% vs. 90% at one month, 44% vs. 72% at four months, and 26% vs. 50% at seven months postpartum. The majority of black women (53%) used formula supplements in hospital, which was the only factor significantly related to a shorter duration in this group ( $p < .01$ ). The high rate of formula supplementation among black women and its strong association with shortened duration of breast feeding point to a

need for more advice and support and less reliance on formula during the hospital stay.

Sociodemographic differences between breast and formula feeders have been extensively studied, yet these factors are not modifiable. Identification of maternal infant-feeding attitudes and mothers' perceptions of social support for breast feeding is important for planning education programs. Three attitudes predictive of breast feeding were identified by factor analysis: "breast feeding is best for the baby", "breast feeding is not socially restrictive", and "maternal confidence in ability to breast feed". Breast compared to formula feeders were more likely to agree that "breast was best" and expressed more confidence in their ability to breast feed. Both breast and formula feeders disagreed that breast feeding allows social freedom; however, formula feeders interpreted this more negatively. Maternal perceptions of social support for breast feeding from five individuals (the baby's father, respondent's mother, closest other relative, closest female friend, and obstetrician) were measured. Breast feeders perceived the baby's father and obstetrician as being most supportive and influential, whereas formula feeders perceived only the obstetrician as being supportive of breast feeding. Stepwise binary regression was used to determine the strength of the association between attitudinal, social support, and sociodemographic variables and the outcome, breast versus formula feeding in the hospital. Approximately 50% of the variance in infant-feeding behavior could be explained by three variables in both white and black women. These were maternal breast-feeding attitude (35%), education (9%), and perceived social support (6%).

The analysis of the data from the Bedouin Infant Feeding Study has been somewhat slower than anticipated due to lack of programming support. Earlier analysis had identified a marked seasonal variation in births among Bedouins in this study population with higher rates of births occurring in the winter and lower rates in the summer. This pattern has been confirmed using vital data of all Bedouin births going back to 1975. In contrast, the data on Jewish births in this region of Israel show very little seasonal variation. Analysis of the data on physical growth obtained at intervals in the follow-up of the Bedouin infants during the first year of life show considerable stunting in lengths characteristic of children in underdeveloped countries. Analyses are in progress to determine the relationship, if any, between stunting and infant feeding practices during the first year of life.

The Prevention Research Program is now in the final phases of obtaining approval for the population-based study of the long-term effects of the ingestion of the chloride-deficient infant formulas, Neomullsoy and Cho-Free, in 1978-79. This study is designed after

the Sarasota, Florida pilot study that surveyed the school population of first and second graders, to identify those children who had ingested the chloride-deficient soy formula and children who had ingested other soy formulas as controls. Intelligence testing of these children revealed significantly lower scores in the exposed population in the areas of general cognitive abilities and quantitative skills. The planned study will follow the same design using a larger population. We are hopeful that several of the large counties adjacent to Washington, DC will participate in the study. We anticipate that data collection for this study will be completed by the end of fiscal year 1988.

Perinatal nutrition will be investigated in the Longitudinal study of Perinatal and Nutritional Epidemiology which was conducted in Guatemala City, Guatemala. The study population (n=17,000) was selected from the Guatemalan Social Security Institute's Ob/Gyn Hospital. This is a 230 bed Ob/Gyn hospital with a tertiary neonatal intensive care unit. Women eligible to receive social security benefits were those that were formally employed and the wives of employed men. Between April 1, 1984 and January 10, 1986 pregnant women who had their first prenatal visit at the hospital's prenatal clinic were enrolled in the study. This was a prospective follow-up study of perinatal nutrition and other risk factors associated with negative pregnancy outcome in a lower middle-class urban population. At this point data are being prepared for analysis at the branch. Several topics will be investigated.

Infants with birth weight <2500 g represent 10-45% of all births in developing countries. Several previous studies have attempted to identify mothers at risk. This study is primarily aimed to produce a simple, empirically developed instrument for the identification of mothers at risk of delivering LBW infants in developing countries. Such an instrument would provide information to detect mothers and children at greater morbidity and mortality associated with LBW. It will also explore the type of medical care that is more related with risk level and lower negative pregnancy outcomes.

#### Other Projects

The Branch has completed a study designed to estimate the cesarean delivery rate in United States hospitals and to determine whether the reasons for the rise in this rate have changed since the NICHD Consensus Development Conference on Cesarean Childbirth in 1979. The cesarean delivery rate steadily increased from 9.1% in 1974 to 14.7% in 1978, and to 21.2% in 1984. One-third of the rise in the cesarean rate from 1974-1978 was due to repeat cesareans, and 9% was due to fetal distress. Since 1978, 47% of the rise in the cesarean rate was attributed to repeat cesareans, and 16% to fetal distress. Less of the recent rise in the cesarean rate was due to dystocia and



breech presentation. The rate of cesarean delivery among those with a previous cesarean was 96%. Deliveries said to be complicated by fetal distress increased from 1% in 1978 to 6% in 1984. The incidence of breech presentation dropped by 18%, which may indicate an increase in the successful use of external cephalic version. Additional efforts should be focused on the diagnostic categories of fetal distress and dystocia, because it is likely that the definitions of these complications are changing to include less severe forms.

The Epidemiology Branch is participating in the NICHD Cooperative Maternal Fetal Medicine Unit and Neonatal Intensive Care Unit Networks which have been created to evaluate therapeutic modalities in the perinatal period, especially those relating to low birth weight. The Branch was responsible for establishing the specifications for the Data Center, as well as determination of the format for data entry. Both networks employ a distributed data entry system. Information will be entered directly on a micro-computer at the study sites eliminating the need to exchange of forms by mail. In addition, the computer will directly aid the collaborating centers in determining eligibility and monitoring protocol compliance. The Epidemiology Branch provides advice to the data center and the Steering Committee of these two networks on epidemiologic and clinical trials issues. The Maternal Fetal Network consists of seven leading obstetrical centers, a data center and representatives of the Epidemiology Branch and the Pregnancy and Perinatology Branch. In one study, scheduled to begin in August, women whose pregnancies have gone beyond 41 completed weeks will be randomized to immediate induction of labor or surveillance and serial tests of fetal well-being with labor being induced only for demonstrated fetal compromise. Neonatal and maternal outcomes will be compared between the two groups. Results of this study will provide insights on ways to reduce the increased neonatal morbidity associated with post dates pregnancies, and possibly to reduce the high Cesarean section rate seen among these women. Further studies are being planned on the use of low-dose aspirin to prevent pre-eclampsia and the use of antibiotics in cases of idiopathic preterm labor.

Within the Neonatal Network the Prevention Research Program has been instrumental in refining the protocol to determine whether or not the prophylactic administration of intravenous immunoglobulin (IVIG) will prevent nosocomial infections in very low birth weight infants. Implementation of this protocol should occur in the fall of 1987. A second protocol being readied for implementation in the Neonatal Network will examine the efficacy of tolazoline, a pulmonary vasodilator, in the treatment of persistent pulmonary hypertension. This disease occurs primarily in term infants who have been asphyxiated at birth or who have suffered from the aspiration of



meconium. The trial of prophylactic vitamin E for the prevention of intraventricular hemorrhage that was mentioned in last year's report continues to undergo refinement and will be considered for implementation when the IVIG protocol is under way.

Since 1984 five cases of Creutzfeld-Jakob disease have been discovered in young adults who received pituitary derived human growth hormone from the National Hormone and Pituitary Program. Because Creutzfeld-Jakob disease is extremely rare in this age group it seems likely that these cases were caused by growth hormone contaminated with the Creutzfeld-Jakob agent. The U.S. Public Health Service has initiated an investigation to locate and interview all recipients of National Pituitary Hormone Program growth hormone to determine the extent of the Creutzfeld-Jakob disease problem. Branch members have assisted in the development of a questionnaire which will provide data on the ultimate stature of growth hormone recipients, their social adjustment and any other treatment associated problems, e.g. slipped capital femoral epiphyses. Design of this questionnaire is now complete. Over 3/4 of the growth hormone recipient cohort has been identified and the project is now proceeding to OMB for clearance.

A national survey is under development to document the health status of children in the U.S. in 1988. Subjects will include accidents, injuries, poisonings, other childhood morbidity, child care, family relationships, perinatal events, use of health services, school performance and behavior. The survey is a collaborative effort of NICHD, the Health Resources and Services Administration, Child Trends Inc., the National Center for Health Statistics and the U.S. Census Bureau. It will be implemented as a supplement to the National Health Interview Survey. The Branch has taken a very active role in developing the instrument.

#### Presentations

1. Klebanoff MA: Mothers and infants birth weight, Tennessee 1979-1984. American Public Health Association, October, 1986.
2. Kurinij N: American Public Health Association, Las Vegas, NV, October, 1986. Breast feeding in a Biracial Population.
3. Cooper LC: American Public Health Association, Las Vegas, NV, October, 1986. Changes in perinatal mortality by race in U.S. cities, 1972-1981.
4. Rhoads GG: Symposium on Diet and Health sponsored by the International Life Sciences Institute, Alvar, Algarve, Portugal, October 1986. Diet and chronic disease: methodologic considerations.

5. Mills JL: Ross Laboratory Conference, Arizona, November, 1986. Lecture on Malformations in infants of diabetic mothers.
6. Cooper LC: Action for Prevention, Williamsburg, VA, December, 1986. Infant mortality, low birth weight and the need for evaluation of smoking cessation programs.
7. Mills JL: Johns Hopkins University, Baltimore, MD, January, 1987. Early fetal losses.
8. Mills JL: Johns Hopkins University, Baltimore, MD, January, 1987. Congenital malformations.
9. Mills JL: University of Pittsburgh, Pittsburgh, PA, February, 1987. Congenital malformations.
10. Mills JL: Johns Hopkins University, Baltimore, MD, April, 1987. Diabetes and congenital malformations.
11. Klebanoff MA: Mother's birth weight and the risk of preterm and small for gestational age birth. Society for Pediatric Research, May, 1987.
12. Mills JL: American Diabetes Association, Indianapolis, IN, June, 1987. Congenital malformations in the Diabetes In Early Pregnancy Study.
13. Mills JL: Teratology Society, Rancho Mirage, CA, June 1987. Chairing a seminar on Diabetes in Pregnancy and delivering a lecture on malformations in diabetic pregnancy.
14. Mills JL: Centers for Disease Control, Atlanta, GA, June 1987. Findings of the Diabetes In Early Pregnancy Study.
15. Kurinij N: Society for Nutrition Education, San Francisco, CA, July, 1987. Ethnic differences in incidence and duration of breast feeding.
16. Overpeck MD: Changes in perinatal mortality in U.S. cities, 1972-1981. Accepted for presentation at the American Statistical Association Joint Statistical meetings, San Francisco, CA, August, 1987.

NICHD Annual Report  
October 1, 1986 through September 30, 1987

Epidemiology Branch

Publications

Shiono, P.H., Klebanoff, M.A., and Berendes, H.W.: Congenital malformations and maternal smoking during pregnancy. Teratology 34: 65-71, 1986.

Shiono, P.H., Klebanoff, M.A.: Ethnic differences in preterm and very preterm births. J. Amer. Pub. Hlth. Assoc. 76: 1317-1321, 1986.

Mills, J.L., Shiono, P.H., Shapiro, L.R., Crawford, P.B., and Rhoads, G.G.: Early growth predicts the timing of puberty in boys: Results of a 14 year nutrition and growth study. J. Pediatrics 109: 543-547, 1986.

Rhoads, G.G.: Use of case-control studies for the evaluation of preventive health care. J. Ambulatory Care Management 9(4): 53-64, 1986.

Kurini, N., Klebanoff, M.A., and Graubard, B.I.: Dietary supplement and food intake in women of childbearing age. J. Amer. Diet. Assoc. 86: 1536-1540, 1986.

Rotter, J., and Mills, J.L.: Diabetes Mellitus. In Berini, Frank, and Paul (Eds.): Clinical Genetics Handbook. New Jersey, Medical Economics, 1986, pp. 227-234.

Marx, S., Vinik, A., Santen, R., Floyd, J., Mills, J., and Green, J.: Multiple endocrine neoplasia type I: Assessment of laboratory tests to screen for the gene in a large kindred. Medicine 65: 226-241, 1986.

Mills, J.L.: Malformations in infants of diabetic mothers. In John Sever (Ed.): Teratogen Update. New York, Alan R. Liss, Inc., 1986, pp. 165-176.

Rhoads, G.G., Dahlen, G., Berg, K., Morton, N.E., and Dannenberg, A.L.: Lp(a) lipoprotein as a risk factor for myocardial infarction. J. Amer. Med. Assoc. 256: 2540-2544, 1986.

Axelsson, M.L., Kurinij, N., and Brinberg, D.: An analysis of the four food groups using multidimensional scaling. J. Nutr. Ed. 18: 265-273, 1986.

Klebanoff, M.A., and Rhoads, G.G.: Collaborative epidemiological research in perinatology. Seminars in Perinatology 10: 169-178, 1986.

Freni-Titulaer, L.W., Cordero, J.F., Haddock, L., Lebron, G., Martinez, R., and Mills, J.L.: Premature thelarche in Puerto Rico: A search for environmental factors. Amer. J. Dis. Children 140: 1263-1267, 1986.

Moessinger, A.C., Mills, J.L., Harley, E.E., Ramakrishnam, R., Berendes, H.W., and Blanc, W.A.: Umbilical cord length in Down's Syndrome. Amer. J. Dis. Children 140: 1276-1277, 1986.

Mills, J.L., and Alexander, D.: Teratogens and "litogens". N. Engl. J. Med. 315: 1234-1236, 1986.

Mills, J.L., and Graubard, B.I.: La controversia sugli effecti del bere moderatamente durante la gravidanza. In Pachi, A., and Astrei, G. (Eds.): Tutela Della Salute Della Gestante e del Concepito. Rome, Acta Medica, 1986, pp. 123-128.

Moss, A.J., Overpeck, M.D., Hendershot, G.E., Hoffman, H.J., and Berendes, H.W.: In: Smoking and Reproductive Health. Littleton, Massachusetts, PSG Publishing Inc., 1987.

Rhoads, G.G.: Reliability of diet measures as chronic disease risk factors. Am. J. Clin. Nutr. 45: 1073-1079, 1987.

Shiono, P.H., Fielden, J.G., McNellis, D., Rhoads, G.G., and Pearse, W.H.: Recent trends in cesarean birth and trial of labor rates in the U.S. J. Amer. Med. Assoc. 257: 494-497, 1987.

Shiono, P.H., McNellis, D., and Rhoads, G.G.: Reasons for the rising cesarean delivery rates: 1970 to 1984. Obstet. Gynecol. 69: 696-700, 1987.

Mills, J.L., Graubard, B.I., and Klebanoff, M.A.: An association of placenta praevia and sex ratio at birth. Brit. Med. J. 294: 544, 1987.

Jovanovic, L., Singh, M., Saxena, B.B., Mills, J.L., Tulchinsky, D., Holmes, L.B., Simpson, J.L., Metzger, B.E., LaBarbera, A., Aarons, J., Van Allen, M.I., and the NICHD-DIEP Study Groups. Verification of early pregnancy tests in a multicentered trial. Proc. Soc. for Exper. Biol. Med. 184: 201-205, 1987.



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Klebanoff, M.A., and Yip, R.: The influence of maternal birth weight on the rate of fetal growth and duration of gestation. J. Pediatrics, in press.

Mills, J.L., and Graubard, B.I.: Is moderate drinking during pregnancy associated with an increased risk for malformations? Pediatrics, in press.

Malloy, M.H., Hartford, R.B., and Kleinman, J.C.: Trends in mortality caused by respiratory distress syndrome in the United States, 1969-1983. Amer. J. Public Health, in press.

Shearer, B., Shiono, P.H., and Rhoads, G.G.: Recent trends in family-centered cesarean birth and women's requests for trial of labor. Birth, in press.

Sweeney, A.M., Meyer, M.R., Aarons, J.H., Mills, J.L., and LaPorte, R.E.: Evaluation of methods for the prospective identification of early fetal losses in environmental epidemiology studies. Amer. J. Epidemiol., in press.

Simpson, J.L., Mills, J.L., Holmes, L.B., Ober, C.L., Aarons, J., Jovanovic, L., Knopp, R.H., and the Diabetes In Early Pregnancy Study: Low fetal loss rates following ultrasound-proved viability in early pregnancy. J. Amer. Med. Assoc., in press.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00318-07 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

A Prospective Study of the Frequency and Duration of Infant Feeding Practices

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Natalie Kurinij Nutritionist EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

.45

## PROFESSIONAL:

.05

## OTHER

.40

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although breastfeeding is generally recognized as the optimal way to feed infants through the first 4-5 months, it is well known that many American women nurse their babies for much more limited periods or not at all. In this prospective study characteristics associated with choice and duration of breast feeding are being investigated. The specific objectives of the study are: (1) to provide detailed information on the change in the infant-feeding pattern over time; (2) to investigate the underlying meaning of the milk insufficiency syndrome; (3) to investigate the relation between maternal employment and choice and duration of breast feeding; (4) to determine the sociocultural differences in infant feeding between two ethnic groups. Approximately 1200 women having their first child in one of three hospitals in the Washington, DC, area were interviewed with respect to factors that may have influenced their infant feeding behavior. Data collection was completed in April, 1986. The initial paper describing socio-demographic factors associated with incidence and duration of breast feeding in black and white women has been accepted for publication, and other analyses are currently underway.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00323-07 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

District of Columbia Perinatal Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Heinz W. Berendes Director PRP/NICHD

COOPERATING UNITS (if any)

Epidemiology Branch, PRP, NICHD (L.C.Cooper); Biometry Branch, PRP, NICHD (D.W.Derman).

LAB/BRANCH

Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). It was necessary to re-key all of the data originally submitted by SRA Technologies. One hundred percent of the data have now been keyed. The data are now being manually edited and cleaned. It is expected that preliminary results will be available by fall, 1987. Complete analysis with results submitted for publication in a peer review journal is expected to begin by late fall, 1987.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00325-06 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

COOPERATING UNITS (if any)

Biometry Branch, PRP, NICHD (H.Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The Epidemiology Branch (EBRP) is conducting a case-control study in Illinois and California to determine whether the use periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect are being ascertained through perinatal networks, vital records, and other sources and are being matched to two controls on maternal race and geographic locale. One control is a mother with a normal pregnancy, and the other the mother of an infant with a fetus with a major health problem. Cases and controls are being interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. The study has been in the field for over a year. In California over 300 sets of NTD cases, normal controls and malformed controls have been identified and interviewed. In Illinois the corresponding figure is 100. This will provide an excellent number of subjects for analysis. Nonetheless, we intend to identify as many cases as possible born before May 1, 1987. This will require identifying new cases through July 1987 and new control subjects through October 1987. We hope to complete the data editing phase of the study and begin analysis by the end of 1987.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00329-05 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Heinz W. Berendes Director PRP/NICHD

## COOPERATING UNITS (if any)

Epidemiology Branch, PRP, NICHD (M.Overpeck and L.C.Cooper); Greater Washington Research Center, Washington, DC (J.Maxwell); Better Babies Project, Washington, DC (D.Coates).

## LAB/BRANCH

Office of the Director, PRP

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL:

1.4

## OTHER

.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Better Babies Project (BBP) pilot study was a three-year research and demonstration effort to reduce the rate of low birth weight and associated infant mortality and illness in a specific high risk area of the District of Columbia. During the pilot phase of the BBP evaluation, Levine Associates, under the NICHD contract, developed computer programs for editing of each data collection form at the time of data entry and the production of basic cross tabs and error reports. NICHD is presently involved in selecting a contractor for the full BBP Research Trial. Levine Associates will continue data management until the new contractor is selected. The Project will attempt to identify all pregnant women in a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findings a number of revisions were made in the forms and interventions. These revised forms and interventions were developed and piloted. A four year trial of the project began September, 1986.

NICHD had let out two contracts for the Better Babies Project to assist with the evaluation. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, provided us information on all pregnant women delivering in the District of Columbia during the period of the project. It is expected that the new contractor for the research trial will be selected by September, 1987 and will complete analyses of preliminary data by June 1991.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00331-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diabetes In Early Pregnancy Project (DIEP)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/PRP/NICHD

COOPERATING UNITS (if any)

Cornell Univ.Med.Center, NY, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ. of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ.of Washington, Seattle,WA (R.Knopp).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.6

PROFESSIONAL:

0.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Diabetes in Early Pregnancy Project has the following objectives: 1) To examine the relationship between maternal diabetic control during organo-genesis and malformations in the offspring. To identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) To compare early fetal loss rates in women with diabetes and control subjects. Signing a contract with an outside computer services corporation, Group Operations, Inc., has permitted the analysis of the DIEP to proceed much more rapidly. It has become apparent since analysis has begun on several topics that additional editing is required. For this reason editing and analysis have proceeded in tandem. We have been fortunate that the study centers have continued to check data as required despite not having paid personnel assigned to this task. Our first major analytic project, fetal loss rates after normal 8 week ultrasound, has been completed and the results submitted for publication. Our second project has been the basic question of malformation rates in the diabetic and control populations. This analysis has reached the stage of being reported at a meeting of the American Diabetes Association and the Teratology Society in June 1987 and a manuscript has been prepared and is currently undergoing revision.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00332-04 EB

## PERIOD COVERED

October 1, 1986 to September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Risk of Adverse Pregnancy Outcome Following Cervicitis during Pregnancy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert P. Nugent Epidemiologist EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

## COOPERATING UNITS (if any)

Johns Hopkins University, Baltimore, MD (B.F.Polk, L.Berlin).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

0.4

## PROFESSIONAL:

0.4

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

All eligible women (age 18 and older) seen in the obstetric clinic at Johns Hopkins University between November 1983 and January 1985 who agreed to participate had their cervix evaluated for signs of inflammation. In addition cultures were taken for a number of aerobic and anaerobic organisms and a sample of cervical mucus was evaluated for the presence of inflammatory cells. The women were interviewed to obtain information on a number of risk factors related to preterm and low birth weight delivery. The women were then followed to delivery to evaluate the effect of cervicitis on preterm or low birth weight delivery. Approximately 800 women participated in this study.

The gram stains have been reviewed by Dr. Sharon Hillier of the University of Washington for signs of cervicitis and bacterial vaginosis. These data are currently being analyzed. A paper presenting the major findings of the study has been submitted for publication. Mycoplasma hominis, Chlamydia trachomatis, heavy smoking and delivery of a previous low birth weight infant were associated with preterm birth. Chlamydia, Candida albicans, maternal smoking and drinking were associated with intrauterine growth retardation.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00333-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Congenital Anomalies and In Vitro Fertilization (IVF)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/PRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH  
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION  
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.2

PROFESSIONAL:

.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

In vitro fertilization has become an increasingly popular method of conception over the past few years. To date no formal study of infants conceived in vitro has been conducted to determine if they are at increased risk for congenital malformations. Dr. Mills and the Epidemiology Branch are conducting a historical prospective study of infants who have been conceived in vitro and matched controls to determine whether in vitro fertilization carries an increased risk for congenital malformations. The Eastern Virginia Medical School, Norfolk, VA, is serving as study and data center for this project (Dr. Fred Wirth, Principal Investigator). Extensive investigations are performed on each in vitro fertilization subject and control subject. These include physical examination, intracranial ultrasound, echocardiography, electrocardiography, and abdominal ultrasound. Patient evaluation has been completed and our goal of 160 participants has been reached. All data from the study have now been computerized and we will begin the editing process in June of 1987. We anticipate writing a report based on our findings by the fall of 1987.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00334-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/PRP/NICHD

Other: George G. Rhoads Chief, Epidemiology Branch PRP/NICHD

## COOPERATING UNITS (if any)

Office of the Director, PRP, NICHD (H.W.Berendes); University Hospital, Uppsala, Sweden (O.Meirik); Centers for Disease Control, Atlanta (R. Yip); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

.65

## PROFESSIONAL:

.65

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The original description of the association of maternal and infant birth weights was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In a related study, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small for gestational age infant as were women weighing 4000-4499 grams, but the low birth weight women were less than twice as likely to have a preterm infant.

Follow-up of girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently being organized in order to examine their reproductive histories. Small for gestational age, preterm and control girls will be located and interviewed. Hospital records of their confinements will also be retrieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00340-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ethnic Differences in Birth Weight and Length of Gestation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD  
Natalie Kurinij Nutritionist EB/PRP/NICHD

COOPERATING UNITS (If any)

Columbia University (V.Rauh and M.Rosen); Northwestern University (R.Depp and D. Zuskar); Office of the Director, PRP (H.Berendes); Division of Maternal and Child Health, HRSA (S.S.Kessel).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall goal of this project is to define previously undescribed risk factors affecting birth outcome from pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, and Whites. These groups are known to differ in their rates of low birth weight. An extensive questionnaire is under development to explore the cultural, psycho-social, nutritional and other differences among pregnant women from these groups. Pretesting of the interview instruments will take place in the summer of 1987 and interviewing is scheduled to begin in New York and Chicago in the fall. Approximately 200-400 pregnant women in each group will be enrolled and followed to delivery.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00341-04 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cesarean Childbirth Rates in the U.S.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

## COOPERATING UNITS (if any)

Center for Research for Mothers and Children (D.McNellis); American College of Obstetricians and Gynecologists (ACOG), Washington, DC (W.H.Pearse).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

0.15

## PROFESSIONAL:

0.15

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. A nationally representative survey has been completed to determine the cesarean childbirth rates in the U.S. and current hospital policies regarding cesarean childbirth. Members of the EB staff acted as consultants to ACOG to assist in the design of the survey, sampling methodology and analysis of the results. The purpose of the survey was to evaluate changes since 1979 in rates of cesarean delivery and trial of labor after a previous cesarean. Questionnaires were mailed to 538 hospitals and 87% responded. In 1979, 2.1% of women with a prior cesarean birth were given a trial of labor. By 1984, the rate increased four-fold to 8.0%. Over 50% of the trials of labor resulted in a successful vaginal delivery. However, the fraction of hospitals with no trials of labor remains high (54%).

B. In addition to this survey, NICHD has obtained information from the Commission on Professional and Hospital Activities on national rates of cesarean childbirth. The cesarean delivery rate steadily increased from 9.1% in 1974 to 14.7% in 1978, and to 21.2% in 1984. One-third of the rise in the cesarean rate from 1974-1978 was due to repeat cesareans, and 9% was due to fetal distress. Since 1978, 47% of the rise in the cesarean rate was attributed to repeat cesareans, and 16% to fetal distress. Less of the recent rise in the cesarean rate was due to dystocia and breech presentation. The rate of cesarean delivery among those with a previous cesarean was 96%.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00343-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.W. Berendes Director, PRP NICHD

## COOPERATING UNITS (if any)

Department of International Health, Johns Hopkins University, Baltimore, Md. (M.R. Forman); BB, PRP, NICHD (B. Graubard); Computer Sciences Section, PRP (E. Harley); Ben Gurion University on the Negev, Beer Sheva, Israel (L. Naggan)

## LAB/BRANCH

Office of the Director, PRP

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.2

## PROFESSIONAL:

.1

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a study of infant feeding practices among Bedouin tribes residing in the Negev, Israel. The objectives are: the evaluation of changes in infant feeding practices during the first year of life and their relationship to physical growth of children and on gastrointestinal and respiratory diseases during the first year of life.

The information obtained covers 5,000 mother-infant pairs. Two samples have been identified, one was identified at birth and a subsample of these births was followed for a period of 5-8 months. Another sample of children was identified at 6 months of age and followed prospectively to 18 months of age.

The data collection is complete and the data has been computerized by the Ben Gurion University staff. Planning for the analysis and using the tapes have identified a number of problems in the use of the tapes which required resolution and involved very frequent interaction with staff at Ben Gurion University. This and the lack of programming support has slowed down the analysis. Ongoing analyses are focusing on seasonality of births in the Bedouin population and possible explanations of these, the study of the relationship between infant feeding patterns and physical growth and morbidity during the first year of life, especially an attempt to see whether the observed stunting in lengths among these Bedouin children, characteristic of third world children, is related to infant feeding practices.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00344-04 EB

PERIOD COVERED October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Long Term Health Effects of Infant Formulas Deficient in Chloride

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael H. Malloy Research Medical Officer EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

COOPERATING UNITS (if any)

Office of the Director, PRP (H.Berendes); Biometry Branch, PRP, NICHD, (B.I. Graubard); Center for Research for Mothers and Children, NICHD (A.Willoughby).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL:

1.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During 1978 and 1979 two infant formulas deficient in chloride were marketed in the United States. It has been estimated that a minimum of 20,000 infant years of these formulas were purchased and more than 100 children were reported to the Centers for Disease Control with metabolic and other abnormalities, principally hypochloremic metabolic alkalosis. In a study of 21 of these children at 2 years of age a significant inverse correlation between length of exclusive use of defective formula and cognitive outcome as measured by the Bayley Scales of Infant Development ( $r = -.55$ ,  $p = .01$ ) was noted in 21 children. Inverse relationships were also noted with respect to several components of the McCarthy Scales of Children's Abilities at 4 years of age. In a population based study which ascertained the infant formulas used by first and second graders attending public school in one county in the southeastern United States, those who were exposed to defective formula were matched to children who ingested chloride sufficient soy based formulas as infants. Matching variables included age, race, sex, socioeconomic status and birth weight. Those who as infants ingested the defective formula scored lower on the general cognitive index and the quantitative scale (McCarthy) than did the children who used other soy formulas.

To substantiate these findings a further study of children is being initiated in the metropolitan Washington, D.C. area schools. It is anticipated that about 250 children exposed to deficient formula and 500 matched control children exposed to other soy formulas will be recruited. In addition, approximately 50 children with a documented history of hypochloremic metabolic alkalosis resulting from defective formula use will be brought to the Washington area. The performance of all these children on a battery of psychological tests will be measured and a careful statistical analysis undertaken to look for an effect of exposure to defective formula with and without documented illness.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00346-03 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Time Trends in the Incidence of Biliary Atresia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/PRP/NICHD

## COOPERATING UNITS (if any)

Case Western Reserve University (B.Chatterjee)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

0.05

## PROFESSIONAL:

0.05

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project will gather birth certificates and other information on all cases occurring among children born over a period of 12 years in Ohio. Cases will be compared to the other births in the state for evidence of changes in incidence and clustering.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00352-02 EB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Human Immunodeficiency Virus - Related Problems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George G. Rhoads Branch Chief EB/PRP/NICHD

COOPERATING UNITS (# any)

Center for Research for Mothers and Children, NICHD (A.Willoughby); Office of the Director, PRP, NICHD (H.W.Berendes); Family Studies Section, NCI (J.Goedert)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A study of perinatal transmission of HIV and the effect of the virus on infected infants is continuing at SUNY/Brooklyn and has recently been expanded to include Albert Einstein Medical Center. Preliminary findings among drug-abusing women suggest that seropositive gravidae (n=14) are more likely to have unexplained fevers, weight loss, sweats, diarrhea, and/or adenopathy than were seronegative drug abusers (n=21). Mean birth weights were 2612 grams and 2877 grams, respectively. In a separate comparison developmental testing was carried out in 18 seropositive infants and 28 seronegative infants born to women attending a substance abuse clinic or a Haitian obstetric clinic. There were no differences in the Einstein scale at one month of age, but at three months mental development scores (Bayley) were lower in the positive group (mean = 101) than in the controls (mean = 109;  $p < .05$ ). At a mean follow-up of 4.4 months (range 0-12 months) about 40% of infants born to seropositive mothers had some clinical findings related to HIV infection. Recruitment of additional mother-infant pairs is continuing.

Additional initiatives in HIV infection include plans to initiate a randomized study of intravenous immunoglobulins in the treatment of infants who are seropositive for HIV infection. Symptomatic and pre-symptomatic infants will be randomized separately.

A study of the knowledge, attitudes and behavior of health care workers toward AIDS and AIDS patients was completed and indicated the need for more in-service training of hospital workers.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00360-01 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Prospective Study of 1st Trimester Use of Bendectin and Malformations

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/PRP/NICHD

Others: Mark A. Klebanoff Senior Staff Fellow EB/PRP/NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

In this prospective study, 31,602 women were asked at their first prenatal visit about the use of Bendectin; 2,711 women reported use in the first trimester. The odds ratio for major malformations was 1.05(0.78 -1.40). When individual malformations were evaluated, Bendectin use was statistically associated with microcephaly (5.33(1.61-17.7)), cataract (5.33(0.98-29.1)), and lung malformations (4.58(1.76-11.9)). Since it is not clear whether these associations are due to the use of Bendectin or to the indication (vomiting) for which the drug was prescribed, the association between vomiting and these malformations was studied using previously published data from the Collaborative Perinatal Project. In that study, vomiting was associated with microcephaly (3.3(1.1,9.8)) and cataract (3.5(0.8-16.1)). Vomiting was associated with these 2 malformations only among nonusers of Bendectin. Lung malformations were not associated with vomiting during pregnancy (1.3(0.8-2.1)). These data suggest that Bendectin does not cause these malformations; however, the possibility that vomiting is associated with microcephaly and cataract is supported.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00361-01 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Child Health Supplement to the 1988 National Health Interview Survey

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary D. Overpeck Health Statistician EB/PRP/NICHD;

Other: George G. Rhoads Branch Chief EB/PRP/NICHD

## COOPERATING UNITS (if any)

Biometry Branch, PRP, NICHD (H.J.Hoffman); HLBBBranch, CRMC, NICHD (P.C.Scheidt);  
DBSB, CPR, NICHD (V.S.Cain, W.Baldwin); National Center for Health Statistics;  
Bureau of the Census; Maternal and Child Health, HRSA; Child Trends, Inc.(N.Zill)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This survey provides data on a nationwide representative sample of 20,000 children. Subjects include child care, family relationships, accidents, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It will provide data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey will be conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00362-01 EB

PERIOD COVERED April 1, 1987 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Aspects of Perinatal Epidemiology in Central America

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jose Villar

Expert

EB/PRP/NICHD

COOPERATING UNITS (if any)

Computer Sciences Section, PRP, NICHD (E.E.Harley); Biometry Branch, PRP, NICHD (H.J.Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This study attempts primarily to develop a simple instrument, empirically produced for the identification of mothers at risk of delivering a LBW infant. Longitudinal data are available for selecting variables at different points during pregnancy. Sample size of the total population is 17,000. The risk score will be developed in a random sample of 8000 patients and tested in the remaining group. Furthermore, the following projects will be also developed using this source of data:

- Epidemiology of subgroups of IUGR infants and their neonatal morbidity.
- Physical activity and work during pregnancy and pregnancy outcome.
- Protozoan and helminthic infections during pregnancy and its effect on birth weight.
- Lead and calcium metabolism during pregnancy: A longitudinal study
- Lactose malabsorption during pregnancy: A longitudinal study
- Body composition and physical activity during pregnancy and birth weight.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00832-04 EB

## PERIOD COVERED

October 1, 1986 through September 30, 1987

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PIs: Leslie C. Cooper Nurse Epidemiologist EB/PRP/NICHD  
Mary D. Overpeck Health Statistician EB/PRP/NICHD

Others: George G. Rhoads Branch Chief EB/PRP/NICHD

## COOPERATING UNITS (if any)

Office of the Director, PRP, NICHD (H.W.Berendes); Biometry Branch, PRP, NICHD (H.J.Hoffman); Computer Sciences Section, PRP, NICHD (E.E.Harley); Mortality Statistics Branch, DVS, NCHS (H.Rosenberg).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS.

0.6

## PROFESSIONAL:

0.5

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This study reviews changes and differences in perinatal mortality for similar populations over a period of rapid change in technology and medical management of high risk pregnancies.

It will explore whether high rates of neonatal mortality in certain cities can be explained by shifts in mortality from the late fetal to the neonatal period and will compare differences in perinatal experience according to race and city size. A secondary analysis of data sets provided by the National Center for Health Statistics was done based on 100 percent reporting of perinatal deaths. Review of fetal death rates for the periods 20, 24 and 28 weeks gestation and of neonatal deaths for the periods 0-7 and 8-28 days is being used to examine potential reporting differences among cities and shifting of neonatal deaths into the latter period.

These data have not been available publicly for analysis. The analysis should provide an improved standard for comparison of perinatal mortality in differing geographic sites.















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